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FULL PAPER

A microfluidic platform for screening and optimization of organic reactions in droplets

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Abstract

We report an automated microfluidic system for screening and optimization of chemical reactions performed inside microliter liquid droplets. The system offers precise control over generation, merging and flow of droplet "micro-reactors" and over reaction conditions, including the volumes of the reagents, temperature and time. The high level of control coupled with the ability to quickly screen multiple reaction conditions allow us to thoroughly monitor the impact of input parameters on the yield of the reaction. In addition, the reagent consumption is kept remarkably low. As an exemplary use of our system we demonstrate a comprehensive study of acid-catalyzed (*para*-toluenesulfonic acid, *p*-TsOH) model imine formation (condensation of *ortho*-nitrobenzaldehyde and phenylethylamine) in an organic solvent (ethanol). By use of novel screening methods described herein, we unfold that the acid-catalyzed model imine formation in the organic medium can be considered as an assembly of acid-mediated and non-catalyzed reactions, both of which accelerate with increasing temperature.

Keywords Multiphase flow · Droplet on demand · Screening and optimization · Droplet microfluidics · Lab-on-a-chip

Introduction

Microreactors became recently actively investigated for use in research on chemical syntheses [1, 2]. The main goals in the development of modern microreactors for organic synthesis include obtaining the highest possible yield, intensification of chemical processes [3, 4] and assurance of highest possibly standards of safety [5]. Microreactors, mainly due to the extremely fast and efficient mixing and heat transfer [6–10] and tight temperature control [11], help to minimize the amount of substrates and solvents by reducing reaction volumes, increasing the yield, selectivity and efficiency of the reaction. Properly designed microfluidic systems perform chemical processing quicker and more efficiently than conventional macroscopic systems. As a consequence, microreactors are increasingly being used not only in academia but also in industrial applications [12].

Here we describe an automated two-phase microfluidic system developed for screening and optimization of organic reactions. Optimization of multi-parameter conditions of a chemical reaction is important because it allows pinpointing conditions that warrant the highest yield of the products at the lowest cost of synthesis. The ability to screen the conditions of chemical syntheses in miniaturized format is especially important for multi-step syntheses that often require optimization of individual steps. Determining the appropriate reaction conditions such as reaction temperature, time, reagents (catalysts) ratio and concentrations is often needed to maximize the desirable product yield. This applies e.g. to the pharmaceutical

Article Highlights
- A benchtop microfluidic system for chemical screening in microdroplets; made of inexpensive elements; easily reproducible.
- Droplet on Demand encapsulates each individual reaction in a droplet, spending < 1 mg of limiting reagent per reaction.
- Microfluidic screening of model reaction accurately reflects the classical batch conditions.

Pawel Jankowski and Rafał Kutaszewicz contributed equally to this work.

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industry, where large numbers of potentially active compounds need to be generated, initially in very small quantities, as a component of the drug discovery process [13]. Traditional batch techniques used for determining the optimal conditions often require considerable number of experiments and lots of effort [14]. In recent years, robotic systems have been employed to provide a degree of automation when carrying out optimization studies [15]. This approach reduces time and workload, however, it requires expensive equipment and, typically, reagents are added not in the same time, but sequentially. This may cause a necessity of employing arduous series of complex liquid handling protocols including e.g. dispensing, dilution, mixing and sample transport. Moreover, the robotically-actuated pipetting poses a risk of samples cross-contamination.

In turn, biphasic microfluidic platforms dedicated to screening reaction conditions carry a potential to be more efficient and timesaving. Such systems, as compared to conventional robots, could enable a considerable increase of droplet formation frequency. As a consequence, generation of broader droplet libraries with varying volumes, concentrations and/or compositions would become possible. Due to (i) low sensitivity to various fluid physical properties, (ii) possibility of tuning the droplet volume range independently from geometry of microchannels [16] and (iii) the better control over droplet manipulations such as: simultaneous dispensing, processing and transfer, the systems with active droplet generation methods (e.g. on-demand control) are often preferred [17]. We believe that the application of particular interest for automated microfluidic systems is in the multidimensional screening of reaction conditions (e.g. flow rate, temperature, residence time and stoichiometry of reagents).

Until now, the majority of microfluidic systems for chemical syntheses operate with continuous (i.e. single phase) flow [18–22]. The use of droplet "micro-reactors" (microliter volumes) may bring in multiple advantages over continuous flow processes: (i) minimization of reagents consumption by application of microliter-sized plugs, (ii) the possibility of fast changes in the reaction conditions such as concentration and ratios of reagents without additional stabilization of the flow, (iii) generation of internal circulation within segments leading to improved mixing compared to laminar diffusion in continuous flow and (iv) better simulation of batch reaction conditions due to elimination of gradients of concentrations. Nevertheless, the droplet microfluidic systems are relatively rarely used for screening reaction conditions [23]. Reports employing the segmented flow technology for optimization of chemical reactions include, among others, works of Ismagilov et al. [24, 25]. Authors, who presented a study on optimization of the conditions of a deacetylation reaction in droplet microfluidic system, used preloaded cartridges with plugs of hydrolytic reagents to prepare droplets surrounded by fluorinated carrier liquid. Various reagents were tested for selective and efficient ouabain hydrolysis from hexaacetyl to triacetyl form. Wheeler et al. [26, 27] reported optimization of different types of reactions with the use of microwave technology, and again, fluorinated carrier fluid acting as a spacer between the droplet reactors. The application of standard HPLC pumps and valves and a Kawasaki six-axis robot for liquid handling yielded relatively large organic reaction plugs (>200 μL). Davoren et al. [28] presented utilization of segmented flow technology for the rapid measurement of atropisomer racemization kinetics with the use of a very simple device and a system of pumps. Reaction segments were separated by immiscible perfluorodecalin spacers that acted as a physical barrier to minimize diffusion between the plugs. In the last years, Jensen et al. reported automated droplet screening systems for real-time optimization of discrete and, simultaneously, continuous variables in organic reactions. The proof-of-concept work studied the case of 1,2-diaminocyclohexane alkylation [29]. System of such type was later on shown to be capable of exploring several complex optimizations of Suzuki–Miyaura cross-couplings [30]. Afterwards, Jensen and co-workers demonstrated a droplet-based, automated platform for reaction screening and small-scale product isolation. Several types of N–X (X = C, S) bond-forming reactions were performed in an oscillatory flow reactor and a small library of compounds generated [31]. A similar flow chemistry platform equipped with blue LED was subsequently used to study several visible-light photocatalyzed transformations. An additional continuous parameter (LED power) could thus be optimized via flow chemistry approach [32, 33]. Just mentioned systems of Jensen and co-workers all utilize an inert gas as the continuous carrier phase and comprise, among others, automated liquid handler, dual sample loop HPLC injection system, UV multi-wavelength detector and LC-MS spectrometer as well as several six-port, two-way injection valves. Such remarkably high degree of device development makes them available for replication to a limited number of research groups. Recently, an approach different from biphasic segmented flow systems was presented by Perera et al. from Pfizer research group [34]. Authors combined segmented flow and continuous flow approaches by injecting stock reagents directly into a flowing solvent stream. Chemicals were allowed to diffuse together, thus forming monophasic reaction segments which were not separated by any spacer. To avoid cross-contamination, the flux was *in-line* monitored using UV-detection, until the baseline returned to zero. In this way, multiple solvents, bases and ligands were tested in a fixed ratio to find the optimal set of reactants for Suzuki coupling reactions. The system allowed testing over 1500 small-scale variants of the reaction per day. During the screen of admirable number of discrete parameters, the continuous factors such as temperature (100 °C), incubation time (1 min) and flow rate (1 mL/min) were kept constant throughout the study.

Above discussed examples of use of the segmented flow technology dedicated to organic chemistry base on two modes of droplet/segment generation: robotic and passive microfluidic systems. In turn, we present a different approach to droplet generation that bases on droplet-on-demand (DoD) method [17, 23, 35, 36]. We therefore discuss the unique character of our system in the Table 1.

In the current study we demonstrate an automatic microfluidic system dedicated to convenient screening of reaction conditions with the full control over the parameters of in situ generated droplets (DoD) and over droplet handling. The main advantage and distinguishing feature of this method is its simplicity and freedom in generation of multiple combinations of concentrations of substrates by merging fluid streams containing multiple individual reagents into one droplet "micro-reactor". Importantly, the merging process can also be precisely executed in time, yielding a well defined moment of combining reagents and onset of mixing thereof. The system allows for quick optimization of the reaction discrete parameters such as: the ratio of reagents, the optional presence of the catalyst, as well as continuous factors such as the concentration, temperature and reaction time. Because of the use of droplets as "micro-reactors", our setup enables examination of the entire spectrum of conditions consuming minimal amounts of reagents. Droplet-on-demand droplet generation technology, in which we combine all the components of the reaction mixture at the same time, introduces original qualities compared to methods based on robotic autosampling or passive microfluidics. To our knowledge, DoD has not yet been used to optimize organic syntheses. In contrast to the abovementioned literature systems [30–34], our device is fully lab-made, benchtop, takes up much less space, is cheaper, and is constructed with commercially available modules, as it is intended to be conveniently reproduced in other organic chemistry laboratories.

The system is presented on an example of a systematic study on optimization of acid-catalyzed synthesis of (*E*)-1-(2-nitrophenyl)-*N*-phenethylmethanimine — a model imine.

Materials and methods

Materials

We used Fluorinert™ Liquid FC-40 (3 M™) as the continuous phase and ethanol solutions of reagents with or without catalyst as the droplet phase. 0.25 M *o*-nitrobenzaldhyde, 0.25 M phenylethylamine and 0.025 M *p*-toluenesulfonic acid (*p*-TsOH) ethanol solutions contained internal standards for HPLC: naphthalene (20.25 mg · mL^{-1}), anthracene (0.6375 mg · mL^{-1}) and biphenyl (4.375 mg · mL^{-1}), respectively. The internal standards were used to control the correct production of droplets. In non-catalyzed reactions, ethanol

Table 1 Comparison of various parameters of systems dedicated to optimization of organic reactions with different droplet generation modules

Parameters under consideration	Robotic systems [26, 27, 29–31]	Other microfluidic systems [18, 20–22, 24, 25]	Our system
Droplet generation system	Operation on reagents in a sequential mode	Passive droplet generation	Droplet-on-demand (DoD)
The method of controlling the size/volume and composition of droplets	Sequential aspiration of fixed volumes of components (one by one); mixing the sample; droplet generation with the use of injection valve, where droplets size is determined by injection loop	Merging of reagents streams before droplet generation; reagents proportions and droplet sizes controlled by flow rates (mostly with the use of syringe pumps)	Simultaneous merger of all components in precisely fixed proportions; droplet sizes and components proportions are adjusted by valves opening time
System cleaning/system rinsing	Required/necessary	Not required	Not required
Frequency of droplets generation	Generally medium	High; controlled by values of flow rates	High; controlled by valves opening time
Number of droplet components	High	Low; controlled by microchannels construction (mostly not exceed 3)	Relatively high; controlled by connector construction (number of inputs)
Complexity of the apparatus for droplets generation and its cost	Robot, syringe pumps, injection valve High cost	Syringe pumps Low cost	Valves with a controller Low cost
Detection	HPLC, IR, GC MS, spectroscopic methods		HLPC (other methods possible)
Temperature control	Heating baths, thermostated chambers		Peltier module; control in the range of 10 to 70 °C
Reaction time control	Various techniques e.g. using the oscillating movement or manipulation of microchannels length and/or flow rates		By flow manipulation e.g. stop flow technique

free of *p*-TsOH additive, yet still containing 4.375 mg · mL^{-1} of biphenyl internal standard was used. All chemicals were purchased from Sigma–Aldrich unless otherwise specified and used as received. Ethanol (99.8%) and Fluorinert™ Liquid FC-40 were purchased from Chempur (Poland) and 3 M, respectively. Beckman Coulter Solvent Delivery Module 126 with UV-detection (Shimadzu, SPD-6A, wavelength 254 nm) was used to perform HPLC analyses. For the description of the microfluidic system's elements and its construction see Online Resource 1.

The design of the microfluidic screening/optimization setup

Our system comprises four modules (Fig. 1). The first module controls the inflow of all of the liquids. The organic reagents and solvent are deposited in syringes, and the liquids are pushed by the continuous phase fluorinated oil into the second module: a 6-port junction that allows us to merge various input streams and break off a newly formed droplet "micro-reactor" by the on-demand controlled flow of the continuous phase. Once generated, the droplets flow into two mixers (Fig. 1B and C) for thorough mixing of the reagents. The third module — the reactor — allows to precisely control the temperature and time of reaction via stopped flow (Fig. 1D). The last, fourth, module is a phase separator (Fig. 1E). Below, we discuss these modules in detail.

The inlet module

Droplet-on-demand system (DoD) [17] with pneumatic overpressures p_1 and p_2 (Fig. 1) was adopted to dose the fixed volumes of liquids. The use of the DoD with a digital driver enabled controlling the opening and closing of valves (Sirai, Fig. 1A) in a preprogrammed sequence. During formation of each droplet "micro-reactor" the valves were opened (Fig. 2).

To avoid contact between the organic reagents and solvents with the sealing material in the micro-solenoid valves we used a non-aggressive fluorinated oil as 'a liquid piston' [23]. The oil enables dosing of organic reagents from reservoirs.

The middle module — formation of droplets and mixing

Each of the feed lines allowed us to inject a preprogrammed micro-volume of the reagent into the polytetrafluoroethylene (PTFE) cube having five orthogonal inlets and one outlet. In the cube, the inlets fed in a droplet "micro-reactor" with preprogrammed volumes of each reagent. Once generated, the droplet "micro-reactors" (10 × 5 μL per experiment) were broken off and pushed out of the cube by the flow of

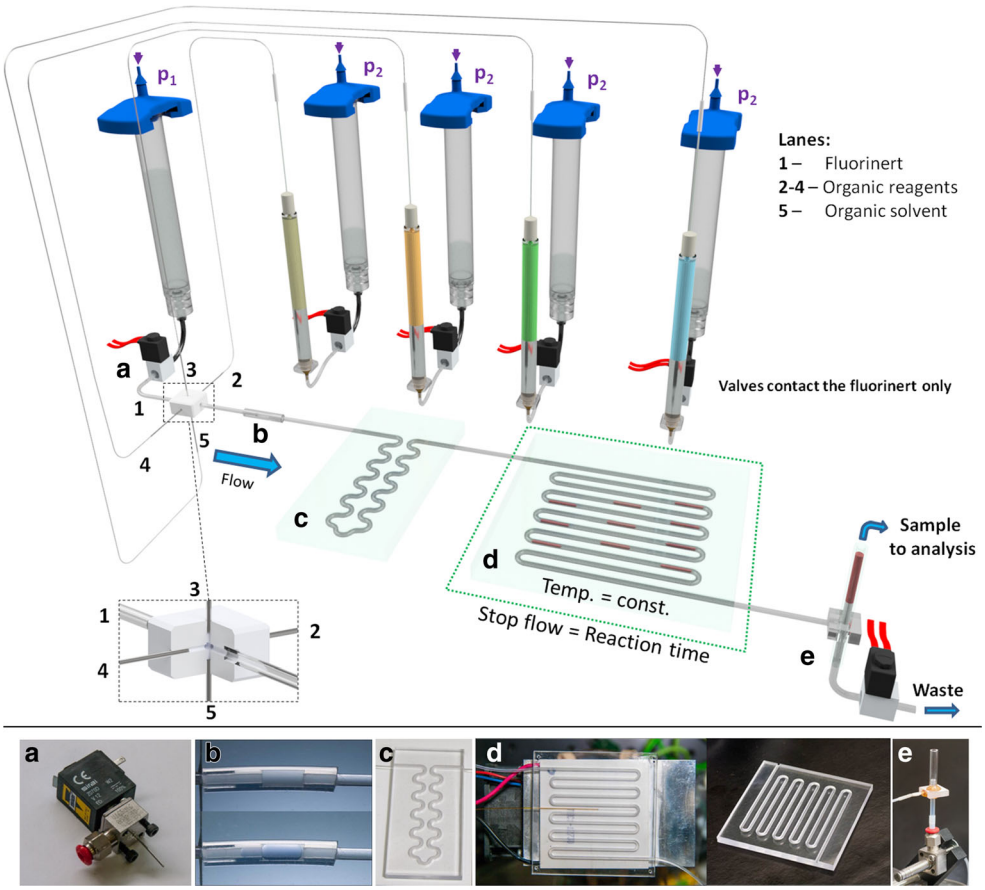


Fig. 1 The complete microfluidic system for screening of chemical reaction conditions in microdroplets. The micro-solenoid valve (*A*), mixers (*B*, *C*), microreactor part (*D*) and phase separator (*E*)

immiscible fluorinated oil (FC-40) in a flow rate of 0.7 mL · min^{-1}, which corresponds to p_1 and p_2 overpressure values of 600 mbar and 500 mbar, respectively. Next, every droplet "micro-reactor" was mixed along in the tube-in-tube mixer (Fig. 1B, Online Resource 3) and thoroughly across in 19.5 cm long meandering channel for rapid advective mixing [37] (Fig. 1C, Online Resource 2) that was connected with the third module, the reactor. The travel time of droplets to the reactor part was equal to 30 s (see Online Resource 2).

The reactor

The reactor comprised 60 cm long fluoroethylene propylene (FEP) tubing (Øint/ext. = 0.8/1.6 mm) lying directly on the thermally stabilized Peltier module. We achieved a temperature control in a range of 10–70 °C by feedback with thermocouple, which was also situated right on the Peltier plate. From above, the FEP reactor tubing was nested in 'rails' (1.6 mm × 1.6 mm, square cross-section) milled in a 5 mm thick polycarbonate (PC) plate (Fig. 1D *right*: PC nest for FEP tubing, *left*: the reactor). One-sided PC nest allowed to spatially fix the tubing and isolated the reaction space thermally from ambient conditions. In case the on-plate temperature measurement indicated a value different from the set temperature, the Peltier plate started heating or cooling (principle of feedback). Such a solution for the temperature control enabled fast reaching and efficient stabilizing (i.e. after up to 3 min from setting) any of reactor temperatures within the range of 10–70 °C, retaining the accuracy of 0.1 °C. Consequently, droplets surrounded by fluorinated oil were entering the pre-heated (or pre-cooled) and thermally stabilized reactor compartment. The heat transfer therein enabled droplet "micro-reactors" to reach the setpoint temperature at the onset of the reaction time (see Online Resouce 1 and Online Resource 2).

We considered the reaction time parameter (plotted on Figs. 5, 6, 8, and 9) as the time when the mixture was already mixed and resided at the assumed temperature. We controlled this time by stopping the flow for a given interval, preset with DoD system (see Fig. 2 and Online Resource 2).

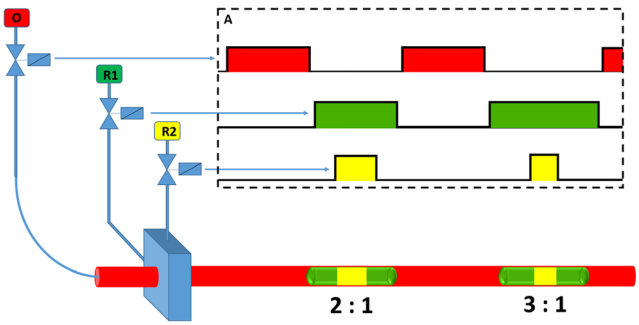


Fig. 2 An operational scheme of the DoD system. The pneumatic overpressure is applied invariably, in the upstream to valves. A digital driver (A) enables controlling the opening and closing of valves in a preprogrammed time sequence of interest. To form a droplet "micro-reactor" containing reagents (R1 and R2), corresponding valves are opened for certain times, correlated to droplet lengths and calibrated prior to use (Fig. 2). Droplets are formed when being broken off and pushed out of the cube by the flow of immiscible fluorinated oil (O). When no valve is opened, the flow stops. Only two lines of reagents are shown in the figure for simplicity

The outlet

Finally, after the reaction, we pushed the droplet "micro-reactors" to the phase separator (Fig. 1E). The separator comprised a stiff vertical tube that separates liquids using their density difference ($\rho_{FC\text{-}40} = 1.85$ g · mL^{-1}, 3 M™, $\rho_{EtOH} = 0.79$ g · mL^{-1}). We collected all ten droplets into the separator to obtain the averaged sample for HPLC analysis.

Analysis

We collected 10 μL of organic phase (top phase in the separator) with a micropipette, diluted it 100-fold in acetonitrile and analyzed with HPLC [38]. The period needed for exit of droplets from the reactor with subsequent reaction quench by dilutive sample preparation was equal to 30 s. A reversed phase C18 column (Kromasil 100) and MeCN/H_2O, 9:1 (*v*/v) with 0.05% vol. triethylamine were used as a HPLC stationary phase and an eluent, respectively. This method with UV detection was a convenient tool for quantitative analysis of the composition of the reaction mixture without any observable hydrolysis of the imine product (see Online Resource 1). Each assay was repeated two or three times and imine molar yield was calculated from the product-to-substrate peak ratio (see Online Resource 1). Despite continuous fluorinated oil imposed a requirement of phase separation prior to the HPLC measurement, it brought significant advantages to the reaction system. Importantly, Fluorinert™ Liquid FC-40 is safe and easy to handle and does not mix, nor react with organic components. It is also mild to the solenoid valves sealing material. In fact, the placement of organic samples in downstream to valves and employing the fluorinated oil as a 'liquid piston' (Fig. 1) allowed us to protect valves form swelling in organic solvents. With its high density, Fluorinert™ Liquid FC-40 was easily separable form ethanolic phase (density difference of 1.06 g · mL^{-1}). Given the high cost of coupling the system to HPLC, (i.e. phase separation, dilution, automatic sampling), samples were collected manually, as we aim in showing a relatively cheap and reproducible by others (in particular: organic chemists) method of screening the reaction conditions.

Results and discussion

Evaluation of analytical parameters of the platform

Firstly, we tested the capabilities of our automated microfluidic system by testing the repeatability, droplet size scalability and accuracy of its droplet-on-demand (DoD) module. With the DoD system we can change the size and frequency of droplet generation in an easy and controlled manner and combine droplets in certain proportions. We present the generated droplet size as a function of the valve opening time in the Fig. 3.

The results show very good linear scalability of a droplet size in the tested time range with very good repeatability for all DoD valves — a linear correlation coefficient was equal to 0.999 for each valve. To test the accuracy of the microfluidic platform (Fig. 4) we used two internal standards (20.25 mg · mL^{-1} of naphthalene (**3**) and 4.375 mg · mL^{-1} biphenyl (**4**)) characterized by different molar absorption coefficients.

Their isovolumetric amounts yield the same values of absorbance, and hence the same peak areas ($S_{naphthalene}/S_{biphenyl}$ = const = 1; Fig. S4). We prepared a number of volumetric ratios of these two standards via DoD valves, mixed them in the tubing and analyzed with an HPLC detector. We confirmed that our system is highly accurate (ca. 2% error, corresponding to the intended volume ratios) in a wide range of concentrations when 8 μL droplets (1600 ms total valve opening time) were generated. We also established the minimum volume of monodisperse droplets that retain the good accuracy (of less than 5% error) to be ca. 4 μL. This corresponds to the obligatory time of opening a single valve for more than 200 ms.

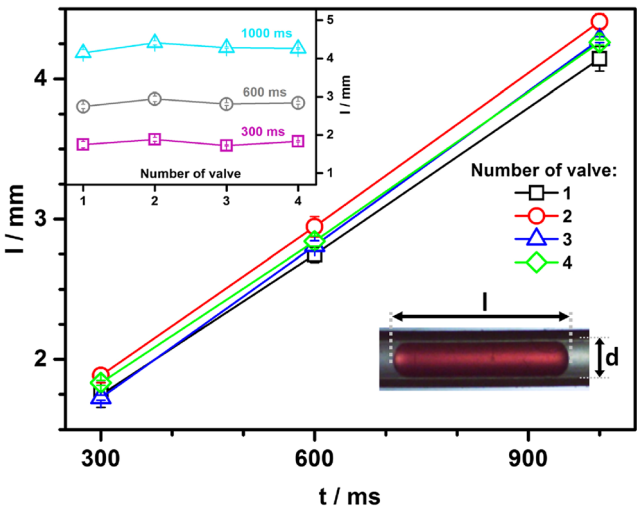


Fig. 3 Mean values of length of droplets as a function of the valve opening time. The lengths were measured via image analysis

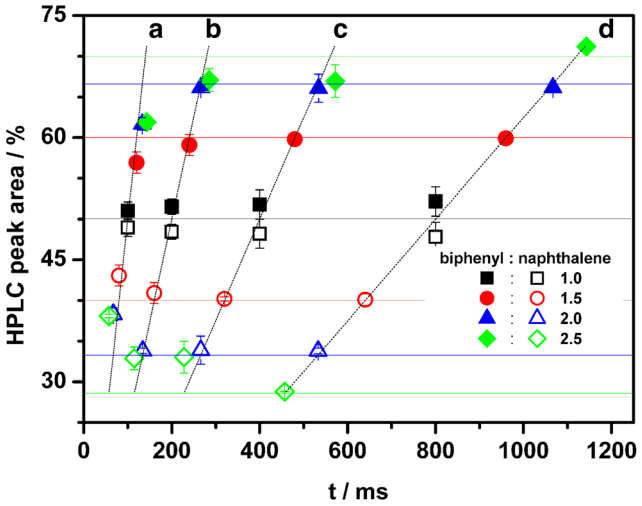
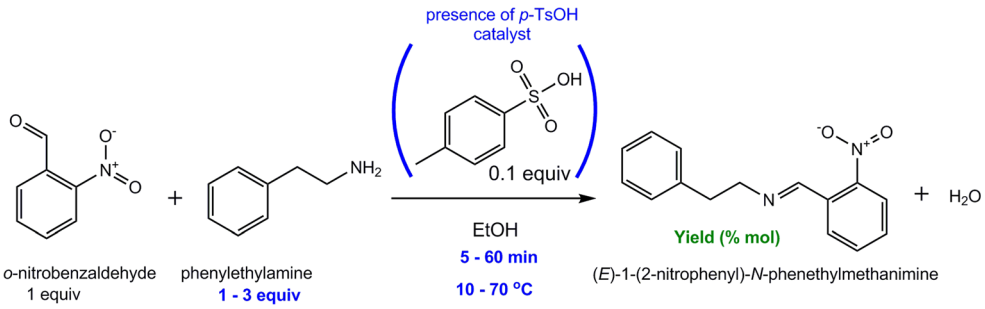


Fig. 4 Available control over the accuracy of formation of organic mixtures of biphenyl with naphthalene (the solutions prepared in molar concentrations resulting in the same HPLC peak areas for the same volumes; peaks 3 and 4 in Fig. S4) in a function of the valve opening time. The total valve opening time was equal to 200 (*A*), 400 (*B*), 800 (*C*) or 1600 ms (*D*), respectively. Colored lines represent the intended volume ratios

Screening of a model reaction: A proof-of-concept application

For a representative example of an application of our microfluidic platform, we demonstrate a four-dimensional screening of a model imine formation — a condensation of *o*-nitrobenzaldehyde and phenylethylamine yielding (*E*)-1-(2-nitrophenyl)-*N*-phenethylmethanimine, in ethanol as a solvent. Microfluidic screening included testing: (i) the ratio of reagents (aldehyde and amine), (ii) temperature, (iii) reaction time and (iv) influence of *p*-toluenesulfonic acid (*p*-TsOH, 10% mol) as a catalyst (Scheme 1).

Our interest in the imine formation was motivated by the wide use of this class of reactions in organic synthesis [39]. Moreover, imine formation reactions are also commonly encountered in enzymatic processes in numerous metabolic pathways in living organisms [40]. The choice of this particular imine formation was dictated by: (i) relatively low reaction rate — a feature that enables easy screening of various reaction parameters and (ii) possibility of simple detection of substrates and product with the use of HPLC. In the literature, a single report on this condensation can be found. Sidhu et al. performed the reaction in ethanol without catalyst and in a fixed time and temperature (24 h, room temperature), reporting a 62% yield [41]. In our experiments we used the same solvent (ethanol) but widened the range of other reaction parameters.



Scheme 1 The model reaction and specification of parameters (marked blue in the figure) tested for their impact on the yield (green) of this reaction

State of the art in the field of imine formation reactions

To our knowledge, an acidic catalysis of the imine formation was a subject of extensive study only in the aqueous environment but not in organic media [42]. It was established that the mechanism is strongly dependent on the value of pH of the aqueous solution. At acidic pH the nucleophilic attack of amine is the rate determining step of the reaction. In turn, higher pH causes the release of water molecule from the tetrahedral hemiaminal intermediate to be the bottleneck. The break-point depends on the nature of amine (pK_a) and both pathways, either at acidic or neutral pH involve ionic intermediates [43]. In turn, following the recent reports by Di Stefano and co-workers [43], little is known about the course of this class of reactions in organic media. There is no evidence for charged intermediates during the imine formation in organic solvents. Rather, it is speculated that the reaction follows through two consecutive four-membered transition states: first — leading to an intermediate hemiaminal, and second — yielding an imine product via decomposition of this intermediate. In each transition state chemical bonds form and break in a concerted fashion and, therefore, ionization does not occur.

Preliminary microfluidic screening

In a preliminary screening, we looked at the effect of reagent ratio in non-catalytic conditions in a wide range of temperatures (10–70 °C) and short reaction times (5–10 min; Fig. 5a and b).

Our goal was to explore if an increase of the molar excess of amine (from 1 to 3 equivalents) would raise the yield of the product. We documented that excess of an amine affects the yield of the reaction to low extent. Nevertheless, given the highest yield was obtained for 3 equiv. of amine to aldehyde, we selected this proportion of substrates for further experiments.

Extended microfluidic screening

With specified ratio of reagents, we tested an effect of *p*-TsOH, (in a fixed, catalytic amount of 10% mol), temperature (10–70 °C) and reaction time (5–60 min). We carried out microfluidic reactions in thus extended range of parameters both still in the absence of the *p*-TsOH ("Non-catalyzed" variant in the Fig. 5c and d), as well as, in parallel, with an addition of *p*-TsOH ethanolic solution ("Catalyzed" variant in the Fig. 5c and d). To maintain the comparable initial concentrations, volumes of droplets in non-catalyzed reactions were respectively filled up with ethanol free of *p*-TsOH.

Reagent consumption

The reaction volume per microfluidic experiment was equal to 50 μL (10 × 5 μL droplet). Given the concentrations we used (i.e. 0.25 M for each substrate), one microfluidic experiment consumed 2.50 μmol of the limiting substrate (*o*-nitrobenzaldehyde). It is worth to underline that the entire microfluidic screening (consisting of 154 original microfluidic experiments, each thrice repeated) consumes then 1.16 mmol of aldehyde (174 mg).

Discussion of results obtained in the microfluidic screening

The results of the entire microfluidic screening (Fig. 5) prove that the reaction yield is a strong and smooth function of both reaction time and temperature. Below, we discuss our results in detail.

Chemoselectivity of the reaction and the product stability

Interestingly, we find that in the presence of catalytic amount of a strong acid (*p*-TsOH $pK_a = -2.8$, water) [44] the possible undesirable acetalization of the aldehyde with ethanolic solvent does not occur (see Online Resource 1). Moreover, formation of this model imine is irreversible and the product is stable in a whole range of tested parameters.

Gain in yield attributed to catalysis

We found that the presence of *p*-TsOH improves significantly the imine yield in all tested conditions (Fig. 6).

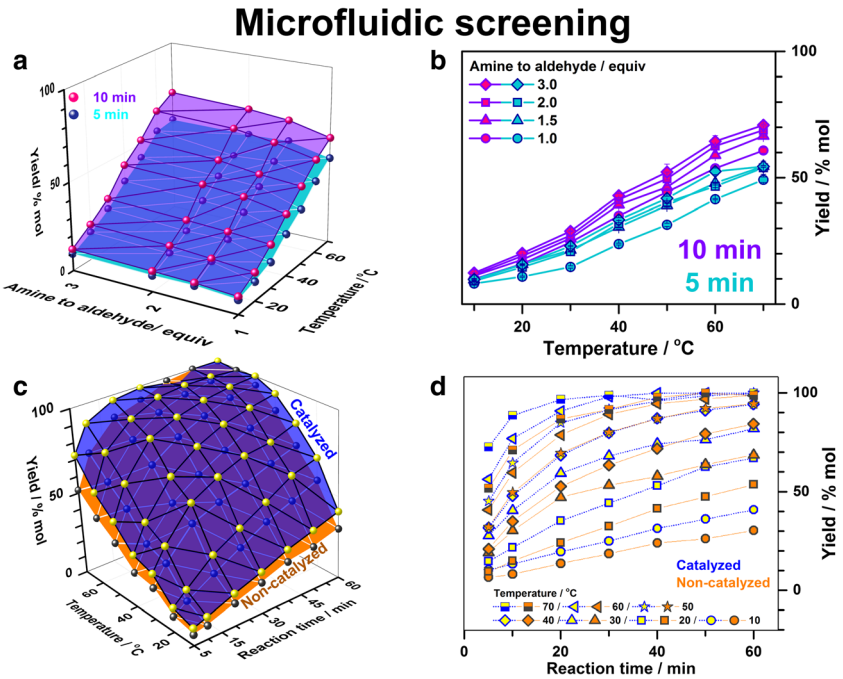


Fig. 5 Results of the microfluidic screening of the condensation of *o*-nitrobenzaldehyde and phenylethylamine in ethanol: **a** optimization of reagents ratio and **c** effect of temperature, reaction time and presence of catalyst and the corresponding two-dimensional graphs **b**, **d**

The maximum of additional imine yield equals to ca. 21% at 70 °C and short (5 min) reaction time. The microfluidic screening revealed that with decreasing temperature the maximal yield gain shifts to the higher reaction time, which is expressed by the diagonal "ridge" on the yield gain surface. This is likely attributed to the character of the process, which involves a fixed amount of starting materials: from the onset of reaction the limiting substrate is consumed faster in the catalyzed variant. At some point of the reaction progress, the concentration of limiting substrate becomes so low, that increasing the conversion lead by the catalyzed reaction is no more possible. Contrary to that, the non-catalyzed variant, although still slower at the molecular level, becomes macroscopically faster and begins to reduce the yield difference. That borderline point corresponds here to 70–80% conversion of the catalyzed process and, for reactions performed at 30–70 °C, does not depend on the temperature. At lower temperatures (10 and 20 °C) the conversion of such height is not reached even in an hour, thus the maximal yield gain occurs about the maximal studied reaction time (60 min).

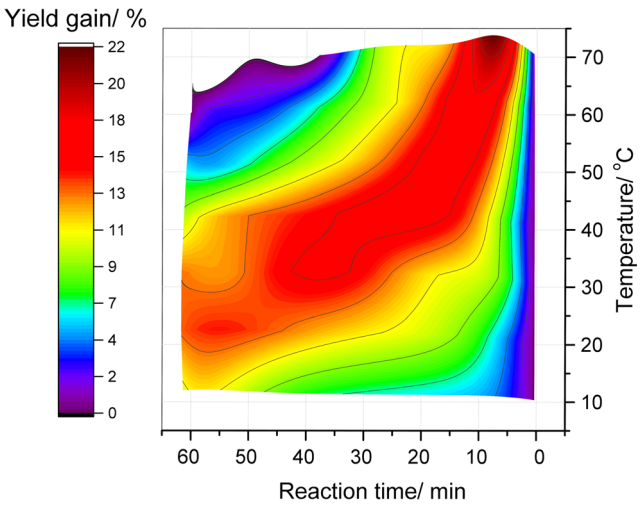


Fig. 6 The gain in yield of the model reaction attributed to catalysis. The continuous two-dimensional graph was obtained by junction of subtracted continuous reaction profiles: Yield gain (%) = $Yield_{cat}$ (%) – $Yield_{Noncat}$ (%). See Online Resource 1 for regression data

Initial turnover frequency of *p*-TsOH catalyst (TOF_0)

Having calculated the instantaneous reaction rates versus time for both catalyzed and non-catalyzed reaction variants at each studied temperature, we could easily determine the initial turnover frequencies of the *p*-TsOH catalyst (TOF_0) in the model reaction (Fig. 7a, see Online Resource 1 for methods).

At the reaction onset, the acidic additive is therefore able to provide 1–12 additional moles of imine per mole *p*-TsOH per minute of reaction time, whereas a relative initial reaction acceleration due to presence of 10% mol *p*-TsOH ($V_{init,Cat}$/$V_{init,Non\text{-}cat}$) is in the range of 1.4–1.8-fold. Noteworthy, the initial turnover frequency of this strong organic acid exhibits a pronounced increase with temperature.

Rate constants estimation

Comparison of experimental data with the integrated rate equation of second order A + B ➔ P reaction allowed us to estimate the values of model reaction rate constants (Table 2, see Online Resource 1 for details). Ranging from 7.4×10^{-4} to 2.4×10^{-2} M^{-1} s^{-1}, the rate constant values, as to the order of

magnitude, are comparable to those measured for the condensation of benzaldehyde and butylamine [45]. We note furthermore, that rate constants of catalyzed reactions are 1.5–2 times greater than these of non-catalyzed counterparts. Importantly, this rate acceleration increases with temperature (Fig. 7b), which harmonizes with same tendency observed for TOF_0 values.

Overall, the microfluidic screening results indicate that the formation of *o*-nitrobenzaldehyde–phenylethylamine-derived imine in ethanol with 10% mol of *p*-TsOH added can be considered as a sum of two processes, namely: non-catalyzed and acid-mediated pathways, both of which accelerate with increasing temperature.

Table 2 Estimated second-order rate constants of model imine formation

Temperature/°C	$k_{Non\text{-}cat}/M^{-1}\ s^{-1}$	$k_{Cat}/M^{-1}\ s^{-1}$
10	7.4×10^{-4}	1.1×10^{-3}
20	1.6×10^{-3}	2.4×10^{-3}
30	2.7×10^{-3}	4.2×10^{-3}
40	4.3×10^{-3}	7.0×10^{-3}
50	7.2×10^{-3}	1.2×10^{-2}
60	1.1×10^{-2}	1.9×10^{-2}
70	1.2×10^{-2}	2.4×10^{-2}

Screen in batch conditions

Next, we compared the operation of our microfluidic setup to a classical organic synthesis approach. We performed a control screen in batch conditions (2.5 mL of reaction mixture): to a flask containing a magnetic stir bar, solutions of aldehyde, catalyst/solvent and amine were added sequentially (same solutions as used in the microfluidic setup). The mixture was stirred in a bath at different temperatures and reaction times. Then, a sample for HPLC was taken, the same way as in the microfluidic experiments. As for the control experiment, we covered about the same range of parameters, but with much smaller resolution in sampling the different values of the parameters (Fig. 8). We emphasize that traditional approach consumed higher amounts of chemicals than in droplet system and required additional manual work for setting up the reactions.

A comparison between batch and microfluidic approach

Superposition of batch and microfluidic results (Fig. 9) shows that microfluidic experiment faithfully reproduces batch conditions and, in this case, can be used as an optimization tool for classical organic synthesis (e.g. in scaling-up). Moreover, the microfluidic system offers more stringent control over studied parameters than batch experiment run with ordinary care. Furthermore, it is quicker and easier to create, or switch between specific reaction compositions as well as to reach and maintain the desired temperature in the microreactor than in batch conditions. Practically, the creation of a different reagent ratio (change in composition) requires only typing in new volumes of droplets. In turn, the thermally stabilized Peltier module enables fast reaching (< 3 min) any of temperatures within the experimental range.

Conclusions

We designed a simple, automated, precise and accurate microfluidic system for screening chemical reactions in microdroplets. We showed its utility by screening over 150 sets of conditions (data points) in acid-catalyzed model imine formation. Our study proposes an easy to set-up and use toolbox for optimization of chemical syntheses. Offering the high level of control over initial mixture composition, temperature, and time of the reaction, the system was able to thoroughly monitor the impact of input parameters on the yield of the reaction. Consequently, instantaneous reaction rates, initial turnover frequencies of the *p*-TsOH, as well as second-order rate constants of catalyzed and non-catalyzed variants could be easily determined. Following the thermal variability of

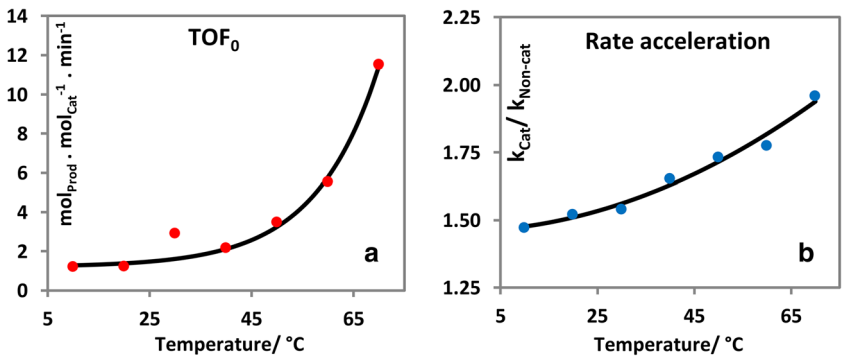


Fig. 7 Analysis of the effect of *p*-TsOH presence in a model reaction; **a**: initial turnover frequency of the catalyst (TOF_0); **b**: comparison of estimated rate constants in catalyzed vs. non-catalyzed reaction variant (Rate acceleration). See Online Resource 1 for calculation methods

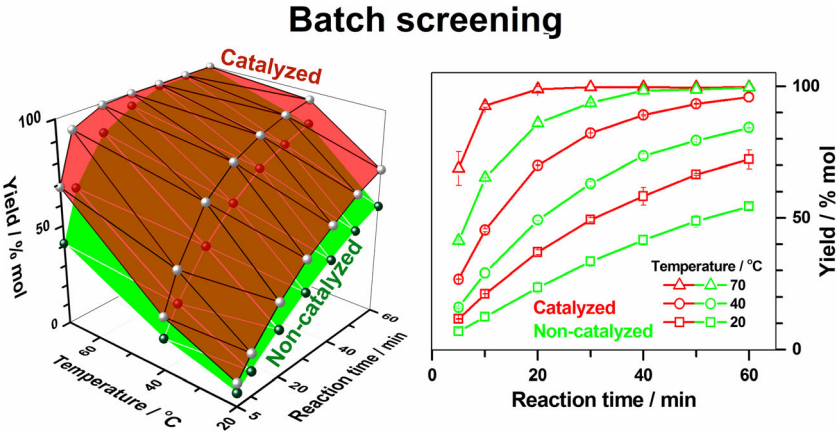


Fig. 8 Results of the batch screening of the condensation of *o*-nitrobenzaldehyde and phenylethylamine in ethanol — effect of: temperature, reaction time and presence of catalyst and the corresponding two-dimensional graph

these kinetic parameters, we conclude that model imine formation with the addition of 10% mol of acid in ethanol can be treated as an assembly of acid-mediated and non-catalyzed pathways, both of which accelerate with increasing temperature. Importantly, results we obtained in microfluidic setup genuinely reflect their batch counterparts. Simplicity of our microfluidic setup allows for its reproduction and utilization in other academic laboratories. It may be particularly useful in catalysis, where an extensive screening is often required on the way to a new, efficient methodology. Similarly, synthetic chemists may likely be interested in the use of such system, especially when only small amounts of reagents are available for optimization of a given synthetic step. Our droplet microfluidic system offers advantages that make it an attractive alternative to both batch and continuous flow approaches to chemical screening and optimization. The level of control over screened parameters we present, as well as automation in setting up consecutive experiments are inaccessible for batch experiment run in a typical way. In turn, our setup distinguishes from many continuous flow systems in aspects of remarkably low consumption of reagents, simplicity and low cost. Noteworthy, processes performed in our setup reach increased safety and become environmentally benign, thanks to low volumes used. Other advantages include the quickness and ease in changing experimental conditions with minimized number of manual operations (i.e. typing valve opening times). Our design can also serve as a module for construction of more complex systems.

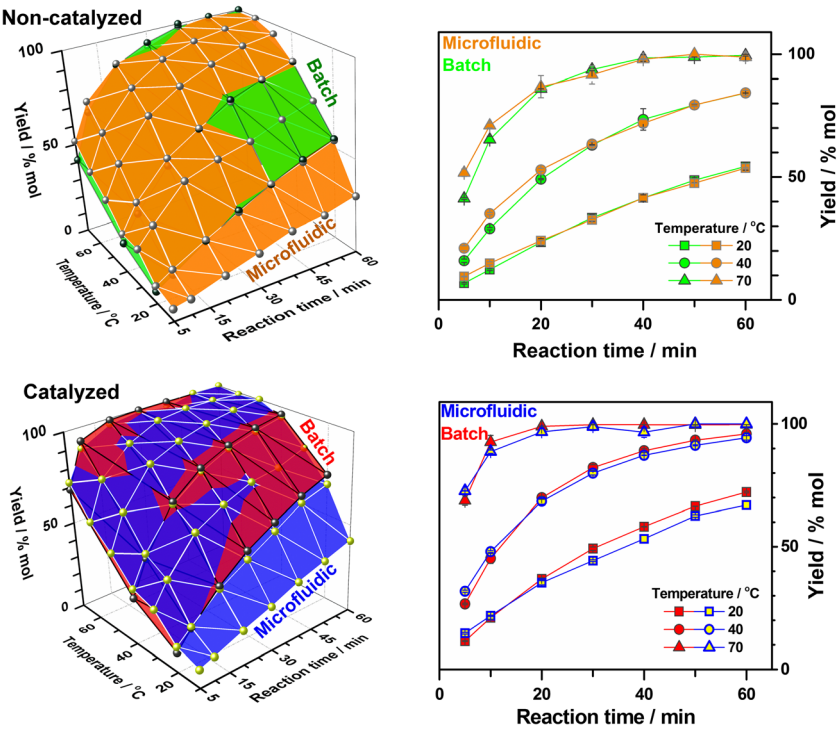


Fig. 9 A comparison of batch and microfluidic screenings of condensation of *o*-nitrobenzaldehyde and phenylethylamine in ethanol

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Compliance with ethical standards

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

References

1. Wirth T (ed) (2013) Microreactors in organic synthesis and catalysis. Wiley-VCH, Weinheim
2. Wiles C, Watts P (2011) Micro reaction technology in organic synthesis. CRC Press, Boca Raton
3. Ramshaw C (1995) The incentive for process intensification. Paper presented at the 1st Intl Conf Proc Intensif for Chem Ind, London
4. Schwalbe T, Autze V, Wille G (2002) Chemical synthesis in microreactors. Chimia 56(11):636–646
5. Stazi F, Cancogni D, Turco L, Westerduin P, Bacchi S (2010) Highly efficient and safe procedure for the synthesis of aryl 1,2,3-triazoles from aromatic amine in a continuous flow reactor. Tetrahedron Lett 51(41):5385–5387
6. Fernandez Rivas D, Cintas P, Gardeniers HJGE (2012) Merging microfluidics and sonochemistry: towards greener and more efficient micro-sono-reactors. Chem Commun 48(89):10935–10947
7. Ji J, Nie L, Qiao L, Li Y, Guo L, Liu B, Yang P, Girault HH (2012) Proteolysis in microfluidic droplets: an approach to interface protein separation and peptide mass spectrometry. Lab Chip 12(15): 2625–2629
8. Lebedev A, Miraghaie R, Kotta K, Ball CE, Zhang J, Buchsbaum MS, Kolb HC, Elizarov A (2013) Batch-reactor microfluidic device: first human use of a microfluidically produced PET radiotracer. Lab Chip 13(1):136–145
9. Watts P, Wiles C (2007) Recent advances in synthetic micro reaction technology. Chem Commun 5:443–467
10. Geyer K, Codee JD, Seeberger PH (2006) Microreactors as tools for synthetic chemists-the chemists round-bottomed flask of the 21st century? Chem Eur J 12(33):8434–8442
11. Schwalbe T, Autze V, Hohmann M, Stirner W (2004) Novel innovation systems for a cellular approach to continuous process chemistry from discovery to market. Org Process Res Dev 8(3):440–454
12. Kumar V, Paraschivoiu M, Nigam KDP (2011) Single-phase fluid flow and mixing in microchannels. Chem Eng Sci 66(7):1329–1373
13. Hochlowski JE, Searle PA, Tu NP, Pan JY, Spanton SG, Djuric SW (2011) An integrated synthesis–purification system to accelerate the generation of compounds in pharmaceutical discovery. J Flow Chem 1(2):56–61
14. Carlson R, Carlson JE (2005) Design and optimization in organic synthesis. Elsevier, Amsterdam
15. Pollard M (2001) Process development automation: an evolutionary approach. Org Process Res Dev 5(3):273–282
16. Guzowski J, Jakiela S, Korczyk PM, Garstecki P (2013) Custom tailoring multiple droplets one-by-one. Lab Chip 13(22):4308–4311
17. Churski K, Korczyk P, Garstecki P (2010) High-throughput automated droplet microfluidic system for screening of reaction conditions. Lab Chip 10(7):816–818
18. Becker R, Koch K, Nieuwland PJ, Rutjes FPJT (2011) Flow chemistry today: practical approaches for optimisation and scale-up. Chim Oggi 29(3):47–49
19. Leung SA, Winkle RF, Wootton RC, deMello AJ (2005) A method for rapid reaction optimisation in continuous-flow microfluidic reactors using online Raman spectroscopic detection. Analyst 130(1): 46–51
20. McMullen JP, Jensen KF (2010) An automated microfluidic system for online optimization in chemical synthesis. Org Process Res Dev 14(5):1169–1176
21. McMullen JP, Stone MT, Buchwald SL, Jensen KF (2010) An integrated microreactor system for self-optimization of a Heck reaction: from micro- to mesoscale flow systems. Angew Chem Int Ed 49(39):7076–7080
22. Moore JS, Jensen KF (2012) Automated multitrajectory method for reaction optimization in a microfluidic system using online IR analysis. Org Process Res Dev 16(8):1409–1415
23. Churski K, Kaminski TS, Jakiela S, Kamysz W, Baranska-Rybak W, Weibel DB, Garstecki P (2012) Rapid screening of antibiotic toxicity in an automated microdroplet system. Lab Chip 12(9): 1629–1637
24. Chen DL, Ismagilov RF (2006) Microfluidic cartridges preloaded with nanoliter plugs of reagents: an alternative to 96-well plates for screening. Curr Opin Chem Biol 10(3):226–231
25. Hatakeyama T, Chen DL, Ismagilov RF (2006) Microgram-scale testing of reaction conditions in solution using nanoliter plugs in microfluidics with detection by MALDI-MS. J Am Chem Soc 128(8):2518–2519
26. Wheeler RC, Benali O, Deal M, Farrant E, MacDonald SJF, Warrington BH (2007) Mesoscale flow chemistry: a plug-flow approach to reaction optimisation. Org Process Res Dev 11(4):704–710
27. Benali O, Deal M, Farrant E, Tapolczay D, Wheeler R (2008) Continuous flow microwave-assisted reaction optimization and scale-up using fluorous spacer technology. Org Process Res Dev 12(5):1007–1011
28. Davoren JE, Bundesmann MW, Yan QT, Collantes EM, Mente S, Nason DM, Gray DL (2012) Measurement of atropisomer racemization kinetics using segmented flow technology. ACS Med Chem Lett 3(5):433–435
29. Reizman BJ, Jensen KF (2015) Simultaneous solvent screening and reaction optimization in microliter slugs. Chem Commun 51(68): 13290–13293
30. Reizman BJ, Wang YM, Buchwald SL, Jensen KF (2016) Suzuki-Miyaura cross-coupling optimization enabled by automated feedback. React Chem Eng 1(6):658–666
31. Hwang YJ, Coley CW, Abolhasani M, Marzinzik AL, Koch G, Spanka C, Lehmann H, Jensen KF (2017) A segmented flow platform for on-demand medicinal chemistry and compound synthesis in oscillating droplets. Chem Commun 53(49):6649–6652
32. Hsieh H-W, Coley CW, Baumgartner LM, Jensen KF, Robinson RI (2018) Photoredox iridium–nickel dual-catalyzed decarboxylative arylation cross-coupling: from batch to continuous flow via self-optimizing segmented flow reactor. Org Process Res Dev 22(4): 542–550
33. Coley CW, Abolhasani M, Lin H, Jensen KF (2017) Material-efficient microfluidic platform for exploratory studies of visible-light photoredox catalysis. Angew Chem Int Ed 56(33):9847–9850
34. Perera D, Tucker JW, Brahmbhatt S, Helal CJ, Chong A, Farrell W, Richardson P, Sach NW (2018) A platform for automated nanomole-scale reaction screening and micromole-scale synthesis in flow. Science 359(6374):429–434
35. Churski K, Nowacki M, Korczyk PM, Garstecki P (2013) Simple modular systems for generation of droplets on demand. Lab Chip 13(18):3689–3697
36. Jakiela S, Debski PR, Dabrowski B, Garstecki P (2014) Generation of nanoliter droplets on demand at Hundred-Hz frequencies. Micromachines 5(4):1002–1011

37. Song H, Tice JD, Ismagilov RF (2003) A microfluidic system for controlling reaction networks in time. Angew Chem Int Ed 42(7): 768–772
38. Lough WJ, Wainer IW (1995) High performance liquid chromatography: fundamental principles and practice. CRC Press
39. Smith MB (2013) March's advanced organic chemistry: reactions, mechanisms, and structure.7th edn. Wiley, New York/Hoboken
40. Hupe DJ (1984) Chapter 8: enzyme reactions involving imine formation. In: Michael IP (ed) New comprehensive biochemistry, vol 6. Elsevier, pp 271–301
41. Sidhu A, Sidhu S, Vineet RM (2011) Synthesis, in vitro antifungal evaluation and structure activity relation of the Schiff bases of some primary amines against Ustilago Tritici. Pestic Res J 23(1):88–95
42. Jones RAY (1979) Physical and mechanistic organic chemistry. Cambridge texts in chemistry and biochemistry1st edn. Cambridge University Press
43. Ciaccia M, Di Stefano S (2015) Mechanisms of imine exchange reactions in organic solvents. Org Biomol Chem 13(3):646–654
44. Guthrie JP (1978) Hydrolysis of esters of oxy acids – Pka values for strong acids – Bronsted relationship for attack of water at methyl – free-energies of hydrolysis of esters of oxy acids – and a linear relationship between free-energy of hydrolysis and Pka holding over a range of 20 Pk units. Can J Chem 56(17):2342–2354
45. Ciaccia M, Cacciapaglia R, Mencarelli P, Mandolini L, Di Stefano S (2013) Fast transimination in organic solvents in the absence of proton and metal catalysts. A key to imine metathesis catalyzed by primary amines under mild conditions. Chem Sci 4(5):2253–2261