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Organic Synthesis in Soft Wall-Free Microreactors: Real-Time Monitoring of Fluorogenic Reactions

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A new approach of "laboratory on a chip" (LOC) devoted to organic synthesis based on electrically operated ionic liquid microdroplets used as air-stable "soft" microreactors was recently introduced. A number of challenging issues have yet to be addressed to allow standard macroscale organic chemistry to be directly transposed in these nonclassical microreactors. In particular, since standard (i.e., magnetical or mechanical) stirring methods are prohibited in such wall-free microreactors, effective alternatives have to be developed to circumvent mass-transfer limitations. With this aim in mind, a fluorogenic version of a click chemistry reaction was developed to evaluate the efficiency of alternative mixing methods on the reaction kinetics. We demonstrate that the combination of chaotic advection created by surface acoustic waves combined with a temperature increase (Marangoni effect) leads to the same kinetics regime as in standard macroscale conditions. This opens the route for application of the new generation of LOC to efficient organic synthesis in microscale.

Laboratory on a chip (LOC) systems dedicated to biotechnological and chemical applications allow the handling of very small quantities of reagents for optimization of reactions while usually producing the desired products faster and in higher yields and purity. Recently, we proposed a new approach of LOC devoted to organic synthesis based on the combination of tiny droplets of Room Temperature Ionic Liquids (RTILs) used as air-stable, "soft" microreactors and electrowetting on dielectric (EWOD)[1,2] as the fluidic motor. This new generation of LOC systems presents several advantages due to the specific features of the two building blocks: (i) ionic liquids exhibit a negligible vapor pressure, which ensures suitable microreactors "bench" lifetime thanks to the lack of evaporation and have been successfully used in many fields, especially in green chemistry and organic synthesis;[3–5] (ii) the EWOD phenomenon allows one to manipulate (move, split, mix) single liquid microdroplets on a two-dimensional electrodes matrix, (by using the electrostatic field pressure applied on the droplet[6–9]), thus avoiding complex fluidic connections and void volumes as observed with standard (i.e., channel) microfluidics[10,11] and offering wider possibilities of combinations than linear channels. Hence microdroplets made of RTILs can be used as "soft" electrically operated microflasks for reagents,[1,12,13] thus allowing chemical reactions to be conducted with such systems. This opens a new promising technological approach for microscale organic synthesis. As a proof of concept, a three-component reaction was carried out in RTIL droplets leading to the expected product after 2 h.[1]

As for other microsystems, the real-time in situ monitoring of the reaction kinetics in such microsystems raises a number of questions. Contrary to standard (i.e., channel) microfluidics, the wall-free microreactors allow for in situ reaction medium sampling, though with inherent experimental uncertainty associated with the sampling of small volumes.[2] In contrast, fluorescence is a noninvasive and quantitative analytic technique, which offers interesting prospects for real-time monitoring of reactions within single microreactors as well as parallel monitoring of many discrete microdroplets by scanning. Such methodology would be of particular interest for various issues including combinatorial synthesis of fluorophores[14] as well as optimization of reaction conditions and protocols. In particular, monitoring and optimization of reagents mixing processes is a critical issue for microfluidics. In particular, since standard (i.e., magnetical or mechanical) stirring methods are prohibited in such wall-free microreactors, effective alternatives have to be developed to circumvent mass-transfer limitations. In this paper, we demonstrate that fluorescence can indeed be used for real-time monitoring of a chemical reaction within single microdroplets, allowing testing and defining experimental conditions for improved mixing efficiency. To achieve this goal, the strategy has been to make use of a fluorogenic

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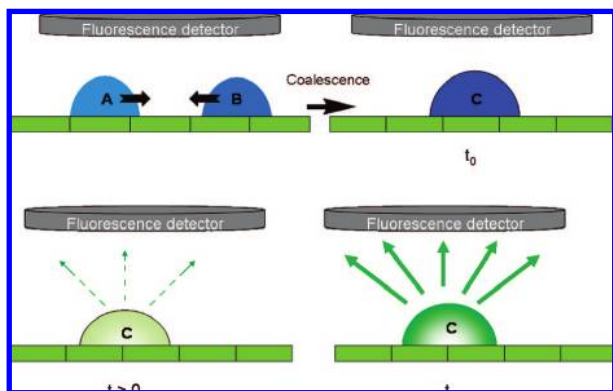(14) Finney, N. S. *Curr. Opin. Chem. Biol.* **2006**, *10*, 238.

Figure 1. Principle of the real-time monitoring of a fluorogenic Huisgen reaction in ionic liquids microreactors handled by EWOD. The two droplets, containing respectively compounds **A** and **B**, were merged, producing fluorescence whose intensity is directly correlated to the generation of final product **C**.

reaction. Recently, authors reported[15–17] efficient synthesizes of fluorescent 1,2,3-triazoles by copper-catalyzed 1−3 dipolar cycloaddition from reaction of azides with terminal acetylenes.[18–20] This well-known so-called click chemistry[21] reaction leads to 1,4-disubstitued 1,2,3-triazoles without byproduct and with excellent yields in various organic solvents, including ionic liquids.[22] In this paper, such a reaction, which can produce fluorescent probes starting from non-fluorescent reagents, was thus implemented in ionic liquid micro-droplets (Figure 1). This was meant to allow for simple real-time monitoring of the reaction by following the fluorescence signal of the droplet—which is directly correlated to the generation of the product—without requiring a complex set of optical filters or acquisitions at different wavelengths to eliminate the contribution of other species to the fluorescence signal. As a first step, the fluorescent monitoring of the formation of 1,4-disubstituted 1,2,3-triazole, **4**, at both the macroscale (flask) and microscale (ionic liquid microdroplets) was demonstrated. With this model reaction in hand, the efficiency of different mixing methods and their influence on the reaction kinetic rates in soft IL microreactors were compared and evaluated. As a result, suitable mixing conditions are proposed as a general protocol for common organic reactions in IL microreactors.

EXPERIMENTAL SECTION

Reagents and Characterization. Ethynylanisole **2** and $Cu(CH_3CN)_4PF_6$ are commercially available from Sigma-Aldrich and were used without further purification. Fluorescent beads (Fluorescent Yellow Green CML latex, reference 2-FY-10000) are commercially available from Interfacial Dynamics Corp. NMR: ^1H chemical shifts (δ) are given in ppm relative to TMS as internal standard, J values in hertz; ^{13}C chemical shifts relative to the central peak of $CDCl_3$ at 77.0 ppm. High-resolution mass spectra measurements and elemental analyses were performed at the Centre Régional de Mesures Physiques de l'Ouest (C.R.M.P.O., Rennes).

Synthesis N-Trimethyl-N-butylammonium bis(trifluoromethylsulfonyl)imide [tmba][NTf₂].

1-Chlorobutane (6.5 mL, 63.1 mmol) and trimethylamine (16.5 mL, 126.2 mmol, 45 wt % aqueous solution) were dissolved in acetonitrile (10 mL) in a sealed flask. The flask was heated to 70 °C overnight. After elimination of volatiles and solvents under vacuum, the residue was washed with diethyl ether and recrystallized from acetone, leading to a white solid. This intermediary salt (9 g, 59.3 mmol) was dissolved in the minimum of distilled water and 1.1 equiv of lithium bis(trifluoromethylsulfonyl)imide (25 g, 87.4 mmol) added. After 2 h of stirring at room temperature, the [tmba][NTf₂] was extracted with methylene chloride and the organic phase dried over Na_2SO_4. Solvent was removed under vacuum giving a water-like free flowing liquid, which was dried under high vacuum at 60 °C for 3 h. Yield: 85%. ^1H NMR (300 MHz, acetone-d_6) δ: 0.95 (t, 3H); 1.40−1.59 (m, 2H); 1.88−2.11 (m, 2H); 3.42 (s, 9H); 3.60−3.75 (m, 2H). ^{13}C NMR (75 MHz, acetone-d_6) δ: 14.1; 20.6; 25.8; 53.9 (t, J_{C-N} = 4.1 Hz); 67.7; 121.8 (q, J_{CF} = 321.0 Hz). ^{19}F NMR (376 MHz, acetone-d_6) δ: −80.3. HRMS (FAB) for $(2C^+, NTf_2^-)^+$: calcd 512.2051, found 512.2069

Synthesis of 1-Azido-4(trifluoromethanesufonyl)benzene **1**. 4-(Tri-fluoromethylsulfonyl)aniline (2.8 g, 11 mmol), water (2 mL), and 2 M HCl (15 mL) were added to a sealed flask. After cooling at 0 °C, 1.0 g of sodium nitrite (14.5 mmol) was added to the solution, incubated for 10 min, and 1.65 g of urea (2.75 mmol) was added. After 1 h at 0 °C, an aqueous solution of sodium azide (1.4 g, 21.5 mmol) and potassium acetate (3.2 g, 32.60 mmol) in water (10 mL) was slowly added at 0 °C. After 2 h at 0 °C, the organic phase was separated, dried over Na_2SO_4, and filtered, and the solvent was removed under vacuum. The obtained oil was purified by chromatography on silica gel (80% heptane/20% ethyl acetate). Yield: 82%. ^1H NMR (300 MHz, $CDCl_3$) δ: 7.27 (d, 2H, ^3J = 8,5 Hz); 8.01 (d, 2H, ^3J = 8.5 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 120.19 (q, $^1J_{C-F}$ = 352.1 Hz); 120.7; 126.9; 133.2; 149.8.

Synthesis of N-*(5-Diethylamino-pentyl)-*N-*trimethylammonium Bistrifluoromethylsulfonimide* **3**. Trimethylamine gas generated by heating at 40 °C a water solution (41 mL, 312.1 mmol, 45 wt % aqueous solution) was condensed, after passing through KOH pellets, in an anhydrous THF dibromopentane (48.1 mL, 200 mmol) solution (100 mL). After 8 h, the ammonium salt was filtered, washed with ether (3 × 25 mL), and dried under high vacuum. This solid was then dissolved in the minimum of distilled water and 1.1 equiv of lithium bis(trifluoromethylsulfonyl)imide added. After 2 h of stirring at room temperature, the solution was extracted with methylene chloride and the organic phase dried over Na_2SO_4. Solvent was removed under vacuum giving a water-like free flowing liquid, which was dried under high vacuum at 60 °C for 3 h. The obtained liquid (36.6 mmol, 17.9 g), ethanol (40 mL), and diethylamine (183.6 mmol, 19 mL of a 40% aqueous solution) were added to a sealed flask. The mixture was heated at 78 °C overnight. After cooling and addition of 146.4 mmol (20.2 g) of solid potassium carbonate, the solution was heated to 78 °C for 4 h. Solvents and excess diethylamine were removed under vacuum. After addition of 50 mL of CH_2Cl_2, filtration to remove

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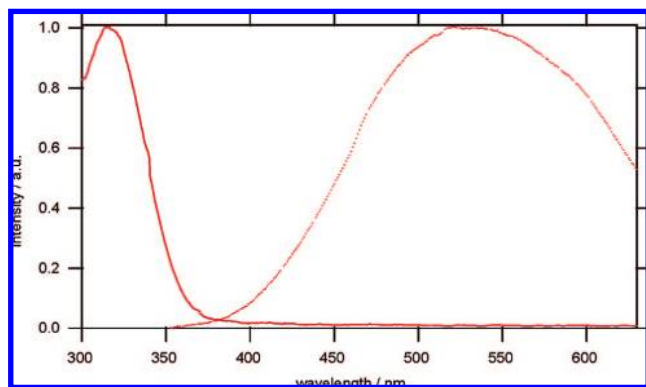(22) Zhao, Y. B.; Yan, Z. Y.; Liang, Y. *Mesult. Tetrahedron Lett.* **2006**, *47*, 1545.

Figure 2. Absorption spectrum (−) and fluorescence emission spectrum (···) of chromophore **4** in [tmba][NTf₂].

KBr and excess K_2CO_3, and washing with water to remove residual K_2CO_3, active charcoal was added and the suspension stirred for 2 h at room temperature and then filtered on a Celite pad. Solvent was then removed under vacuum leading to an oily product. Yield: 60%. ¹H NMR (300 MHz, acetone-d_6) δ: 1.00 (t, 6H, $^3J = 7.0$ Hz); 1.39−1.63 (m, 4H); 1.89−2.16 (m, 2H); 2.38−2.54 (m, 6H); 3.38 (s, 9H); 3.54−3.66 (m, 2H). ¹³C NMR (75 MHz, acetone-d_6) δ: 12.3; 23.8; 25.1; 27.5; 48.1; 53.5; 54.0 (t, $^1J_{C-N} = 4.0$ Hz); 67.9; 124.6 (q, $^1J_{C-F} = 319.9$ Hz). FAB HRMS for $C_{12}H_{29}N_2$ ($M^{+\cdot}$): calcd 201.2331, found 201.233.

Synthesis of 1-Trifluoromethanesulfonyl-4-(4-p-methoxyphenyl-1H-1,2,3-triazol-1-yl)benzene 4 in Droplet. Solution 1: **1** (0.2 mol/L) and $Cu(CH_3CN)_4PF_6$ (0.01 mol/L) are dissolved in [tmba][NTf₂]. Solution 2: **2** (1 mol/L) and **3** (0.03 mol/L) are dissolved in [tmba][NTf₂]. The 0.2-μL droplets of solutions 1 and 2 were deposited on the chip with an Eppendorf micropipet. The droplets were displaced and merged with a tension of 55 V rms. The actuation of the electrodes was switched off and the reaction carried out.

The following data were obtained for pure isolated compound **4** (synthesized in standard macroscale conditions). ¹H NMR (300 MHz, DMSO) δ: 3.83 (s, 3H); 7.11 (d, 2H, $^3J = 8.8$ Hz); 7.90 (d, 2H, $^3J = 8.8$ Hz); 8.44 (m, 4H); 9.50 (s, 1H). ¹³C NMR (75 MHz, DMSO) δ: 55.2; 114.5; 119.0; 121.0; 122.1; 126.8; 127.0; 128.2; 133.1; 142.8; 147.9; 159.5. ESI+/HRMS for $C_{16}H_{12}F_3N_3O_2S$ ($M^{+\cdot}$): calcd 383.05515; found 383.0549. Elemental analysis calcd (%) for $C_{16}H_{12}F_3N_3O_2S$ C 50.13, H 3.16, N 10.96, S 8.36; found C 50.37, H 3.65, N 10.66, S 8.28.

Theoretical Calculations. Natural transitions orbitals[23] of chromophore **4** (Figure 2) were calculated at the TD-B3LYP/6-31G//HF/6-31G level using the Gaussian 98 package.[24] They hint to a clear charge transfer from the electron-donating moiety (methoxyphenyl) to the electron-withdrawing moiety (which includes both the triazole and the trifluoromethanesulfonylphenyl units) that occurs upon excitation.

Absorption and Emission. UV/vis spectra were recorded on a Jasco V-570 double-beam spectrophotometer. Steady-state fluorescence measurements were performed at room temperature using an Edinburgh Instruments (FLS 920) spectrometer working in photon-counting mode, equipped with a quantum counter for excitation correction. Fully corrected emission spectra were obtained at λ_{ex} with $A(\lambda_{ex}) < 0.1$ to minimize internal absorption.

Fluorescence quantum yield was measured using standard methods on air-equilibrated samples at room temperature. Quinine bisulfate in 0.05 M H_2SO_4 ($\Phi = 0.546$) was used as a reference.[25] The reported fluorescence quantum yields are within ±10%.

RESULTS AND DISCUSSION

The 1,2,3-triazole **4** was obtained by a 1,3-dipolar cycloaddition, catalyzed by a copper (I) salt, i.e., $Cu(CH_3CN)_4PF_6$, between azide **1** and the alcyne **2** in [tmba][NTf₂] (*N*-butyl-*N*-trimethylammonium bistrifluoromethylsulfonylimide) in the presence of the supported amine **3** used as a base (Scheme 1).

The macroscale reaction (0.16 mmol, 800 μL of a magnetically stirred solution at 400 rpm) showed a 100% conversion rate after 14 min at 25 °C. Using different temperatures and initial concentrations in **1** (100−500 mM), **2** (100−500 mM), and Cu^I catalyst (2−10 mM), the experimental data were correctly fitted over the whole experimental range using eq 1 as the kinetics rate expression:

$$v = k_0 e(-E_a/RT)[\mathbf{1}]^n[\mathbf{2}]^m[Cu^+]^p \tag{1}$$

where v is the reaction rate, k_0 the pre-exponential factor, E_a the activation energy, n the partial order in reagent **1**, m the partial order in **2**, and p the partial order in Cu$^+$.

Note that the partial orders are averaged over the whole range of reagent concentrations and thus cannot represent a chemical mechanism. However, the values of the partial orders are consistent with previous work, as they fall between the maximal and minimal values obtained by Fediorov et al.[26] in using initial-rate kinetic measurements with different reagents concentrations.

The best fit was obtained for the following values: $k_0 = 1.76$ $L^{0.5}\cdot mol^{-0.5}\cdot s^{-1}$, $E_a = 42$ kJ·mol^{-1}, $n = 0$, $m = 0.6$, and $p = 0.9$. These values indicate that (i) only **2** and Cu$^+$ concentrations affect the kinetic rate and (ii) the temperature influence on the kinetics rate is well accounted for by the Arrhenius law with a high value of E_a showing that the measured kinetics data were not biased by the mass-transfer limitation. As shown in Figure 2, chromophore **4** shows an absorption band in the near-UV region, peaking at 318 nm (extinction coefficient $\varepsilon_{max} = 7.2 \ 10^3$ $M^{-1}\cdot cm^{-1}$). Interestingly, chromophore **4** shows a broad emission band in the visible region—characteristic of a polarized emitting excited state with strong interactions with the environment—peaking at 530 nm (quantum yield $\Phi = 0.15$) in ionic liquid

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Scheme 1. Fluorogenic 1,3 Dipolar Cycloaddition (Click) Reaction Used as a Model Reaction in Ionic Liquid Solvent[a]
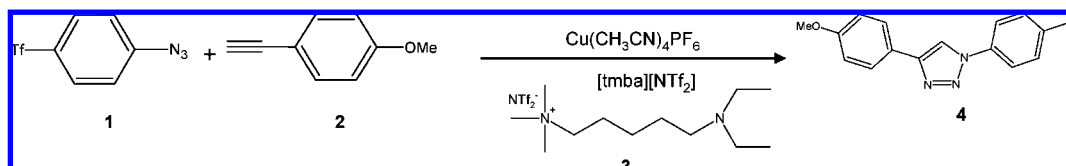
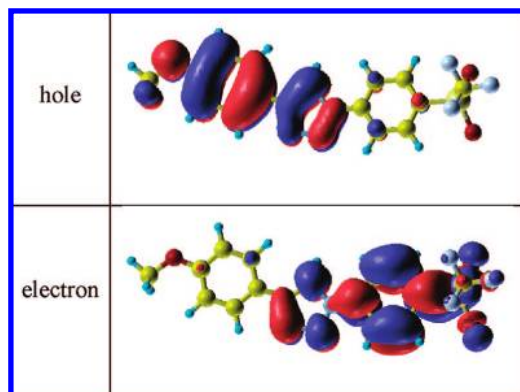[a] The task-specific ionic liquid **3** is used as the base.

Figure 3. Natural transitions orbitals of chromophore **4** calculated at the TD-B3LYP/6-31G//HF/6-31G level using the Gaussian 98 package.
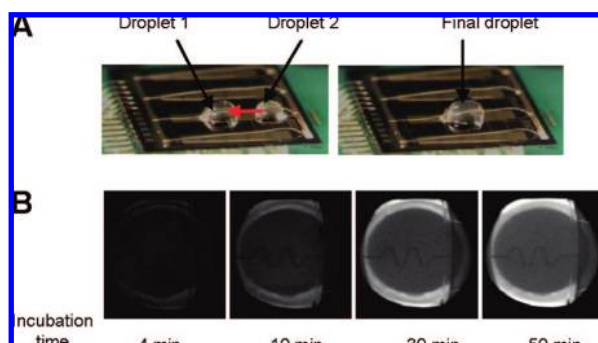

Figure 4. (A) On-chip EWOD merging of microdroplets containing reagents. Droplet 1 (0.2 μL) contains reagent **1** (0.2 mol/L; 10 μg) and catalyst Cu(CH₃CN)₄PF₆ (0.01 mol/L; 0.7 μg) while droplet 2 (0.2 μL) contains reagent **2** (1 mol/L; 26 μg) and base **3** (0.03 mol/L; 2.9 μg). (B) Real time monitoring of the formation of product **4** (microreactor top view).

[tmba][NTf₂] (Figure 2). The corresponding transition can be related to a charge transfer between donor and acceptor units. Indeed, transition orbitals calculations (shown in Figure 3) clearly hint to a charge transfer from an electron-donating moiety (methoxyphenyl) to an electron-withdrawing moiety of the molecule (which includes both the triazole and the trifluoromethane-sulfonylphenyl units) upon excitation. The emission of product **4** in the visible region can thus be used for in situ real-time monitoring of the model click reaction since none of the starting materials are fluorescent.

In order to perform the reaction within a single droplet, a microdroplet of [tmba][NTf₂] containing reagent **1** and catalyst Cu(CH₃CN)₄PF₆ (droplet 1) was converged by EWOD at 55 V toward a second droplet of the same ionic liquid containing reagent **2** and base **3** (Figure 4A).
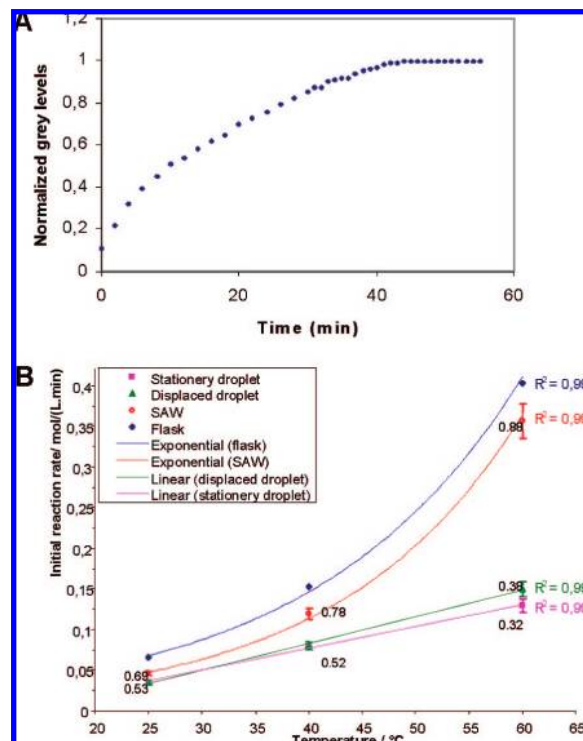

Figure 5. (A) Time evolution of the normalized fluorescence signal generated in the microdroplet reactor by the fluorogenic reaction without mixing activation. (B) Initial reaction rates at different temperatures in the bulk (stirred solution in a flask) and in microdroplets using three different mixing activation methods. The initial reaction rates were determined by recording the fluorescence signal during reaction and by measuring the slope at the origin in each condition. Values of mass-transfer efficiency (ϵ) are indicated.

After coalescence, the fluorescence signal of **4** generated by the reaction was recorded (Figure 4B) and the fluorescent intensities were normalized by the minimum and maximum levels to describe the reaction kinetics (Figure 5A). The fluorescence signal is observed to increase gradually in agreement with ongoing formation of product **4**. A 100% conversion rate is reached after 40 min (as confirmed by HPLC) in the case of a stationary microdroplet. This slow reaction rate, as compared to the rate of the macroscale reaction performed with standard magnetic stirring, is due to the high viscosity of the RTIL used as solvent, i.e., [tmba][NTf₂][27] resulting in slow diffusion of reagents within the microreactor droplet and consequently in diffusion-limited reaction.

To circumvent this critical limitation, different mixing methods were then investigated in order to enhance the molecules' mobility and therefore approach the kinetics rates obtained for macroscale reactions when using standard magnetic stirring. The first mixing

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(27) The [tmba][NTf₂] viscosity with 2950 ppm water was measured to be 81 cP.

method consists of keeping stationary the microdroplet obtained after coalescence and heating it at 40 °C using a resistance kept underneath the EWOD chip in order to create an additional convectional flow and/or a Marangoni effect.[28] Indeed, if the substrate is warmer than the air, the liquid droplet sitting on the substrate chip will experience a warmer region near the substrate and a cooler region near the apex of the droplet. Thus, the surface tension will be lower near the substrate and larger at the apex, resulting in a tangential force on the interface. If sufficient, this tangential force induces a circulation inside the droplet even if the external shape of the droplet does not change. A preliminary experiment with fluorescent beads (Fluorescent Yellow Green CML latex with a 9.8-μm diameter, $\lambda_{exc} = 490$ nm, $\lambda_{em} = 520$ nm), meant to visualize the mixing in the microdroplet, reveals that, above 40 °C, fluorescent beads move along circular trajectories within [tmba][NTf$_2$] droplets (see Supporting Information) and that the beads velocity increases with the temperature.[29] This experiment shows that a simple heating, which produces a temperature gradient and, consequently, a surface tension gradient,[30] can activate mixing in the droplet. The second mixing activation method tested is based on moving the droplet to and fro along a row of five electrodes at a frequency of 0.85 Hz (55 V) creating a flow inside the droplet.[2] For the third mixing activation test, the droplet was positioned on a module generating surface acoustic waves (SAWs from Advalytix AG), which leads to a flow created by the longitudinal pressure wave diffracted inside the droplet.[31,32] It should be noted that the experiment conducted with fluorescent beads shows that, in that case, the mixing process follows a chaotic advection,[33,34] which accelerates the mixing by decreasing the diffusion length.

The model click chemistry reaction was used as a tool to study the influence of these different mixing activation methods on the apparent reaction rate by comparing the initial reaction rates in the microreactor obtained after merging the two initial droplets. The three mixing activation methods lead to a reduction of the time required for reaction completion with different ranges. The fastest reaction completion is obtained under SAW activation, where the reaction is completed within 21 min at room temperature. The initial reaction velocities in droplet, v_{iapp}, determined for the three mixing activation methods were compared to the initial rate v_i determined from a standard macroscale experiment (magnetically stirred solution). In all cases, the measured v_{iapp} values were smaller than v_i indicating that, whatever the mixing activation method, the reaction is mass-transfer limited in the microdroplet. Based on this observation, eq 1 was modified by including a correction factor (ε) that represents the efficiency of the mass transfer (eq 2)

$$v_{iapp} = \varepsilon v_i \tag{2}$$

Efficiency factors of 0.53, 0.53, and 0.69 were obtained at 25 °C for respectively the first (heating), second (to and fro move), and third (SAW) mixing activation methods.

Finally, the three mixing activation methods were studied at different temperatures (Figure 5B). Whatever the temperature, the fastest initial reaction rates is obtained under SAW activation while stationary or displaced droplets exhibit comparatively poor results, especially at 60 °C. Moreover, the initial reaction velocities obtained with stationary or displaced droplet being quite similar, we can assume that the benefit of mixing activation by droplet displacement is moderate. In contrast, whatever the mixing method, the initial reaction rate significantly increases with the temperature, demonstrating that mixing is accelerated by heating. Interestingly, whereas linear increases are obtained with stationary or displaced droplets, an exponential increase is obtained with SAW activation as is the case for standard macroscale reaction. In addition, the reaction becomes less and less mass-transfer limited as the temperature increases with ϵ values approaching 1 (at 60 °C, $\epsilon = 0.88$). This is not the case with stationary or displaced droplet for which temperature increases at opposite lead to decreasing ϵ values (Figure 5). The present study thus clearly shows that SAW activation offers, for soft IL microreactors, an efficient substitute to common macroscale magnetic stirring. This is an important step in the implementation of general and efficient operating protocols, demonstrating that electrically operated IL microdroplets indeed open a promising route toward microscale organic synthesis.

CONCLUSSION

This paper describes the synthesis and the real-time monitoring of 1,2,3-triazole by a 1,3-dipolar cycloaddition in ionic liquid droplets acting as soft wall-free microreactors. This click chemistry reaction was used as a powerful tool to evaluate the efficiency of different mixing activation methods on the reaction progress by comparing the initial reaction velocities. This study provided demonstration that the combination of chaotic advection created by SAW and temperature increase allows us to approach a kinetic regime similar to that for macroscale standard organic reactions in solution (i.e., magnetically stirred solutions of organic reagents in regular "hard" flasks).

SUPPORTING INFORMATION AVAILABLE

Description and fabrication of the EWOD chip; instrumentation and optical setup for monitoring; visualization of Marangoni effect. This material is available free of charge via the Internet at http://pubs.acs.org.

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