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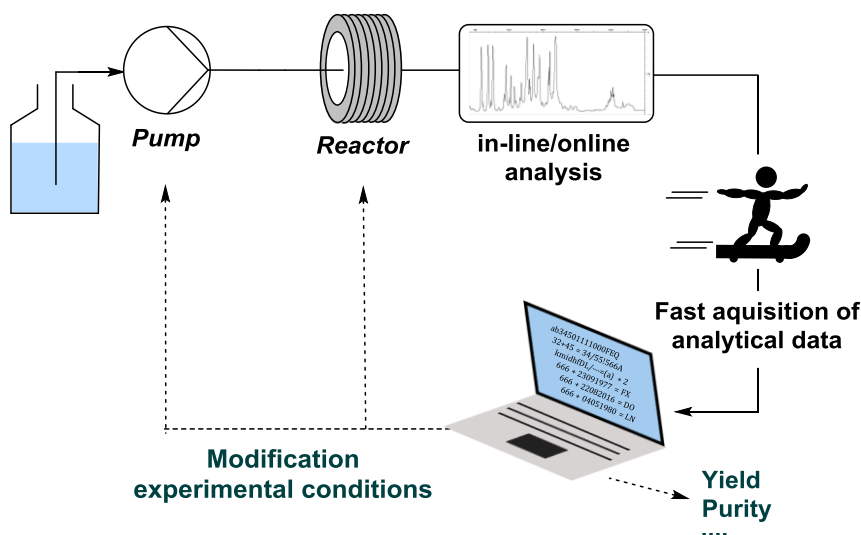
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# Analytical Tools Integrated in Continuous Flow Reactors: Which One for What For?

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## ■ ABSTRACT GRAPHIC



## ■ ABSTRACT

The concept and practices of chemical synthesis are being profoundly transformed toward the development of fully autonomous continuous processes. Critical to the development of autonomous continuous processes is the efficient monitoring of the reaction composition and product quality by in-line and online analyses. The in-line/online acquisition of analytical data allows to monitor at regular interval the reaction composition including hazardous or air sensitive intermediates with the possibility of adapting reaction parameters or interrupt the flow process when a chemical or technical failure is detected. This review presents the main in-line/online analytical tools which can be integrated into flow reactors for the monitoring of chemical reactions. This contribution is more a guide at the service of synthetic chemists

illustrated by selected published examples from leading research laboratories than an exhaustive list of published articles. Ultimately, we would like this review to be an answer to the following recurrent, yet complex, question: “Which is/are the most suitable analytical solution(s) to monitor my chemical reaction?”.

## ■ KEYWORDS

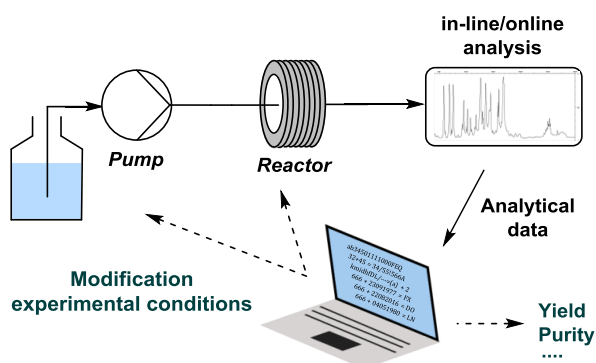
Automation • Chemistry 4.0 • Continuous flow reactor • Flow chemistry •

In-line/Online analysis

## ■ INTRODUCTION

Fine chemical industries and organic synthesis research laboratories are facing a digital revolution which is going to profoundly modify the way chemists think and perform chemical transformations.<sup>1-9</sup> Chemistry 4.0, which has been coined to define the chemistry of the fourth industrial revolution, massively takes advantage of integrated information technology systems, algorithms, and automated machines to reinvent the concepts of synthesis and production established after the Second World War.<sup>10-16</sup> Both research laboratories and multipurpose plants 4.0 will be miniaturized, highly modular, (semi)autonomous and connected, increasing safety and sustainability (smaller size) and reducing cost (modularity and autonomy).<sup>17-19</sup> To reach the objectives of Chemistry 4.0, chemical synthesis will mainly operate in continuous flow mode as this technology offers enhanced safety, better reaction control, “on the fly” monitoring, and easier automation compared to traditional batch reactors.<sup>15, 20-25</sup> Moreover, from a more fundamental point of view, flow chemistry offers the possibility to access “forbidden” transformations as it allows to explore new process windows and also strongly accelerates kinetics through improved heat and mass transfer.<sup>26-27</sup> Critical to the control of continuous processes is the efficient monitoring of process parameters and product quality by process analytical technology (PAT).<sup>28</sup> At the initiative of the United States Food and Drug Administration (USFDA), PAT was initially introduced in the pharmaceutical industry to enhance and control manufacturing processes with the aim of achieving Quality-by-Design (QbD). In the large range of tools covered by PAT, in-line and online analyses of continuous processes have a prominent role.<sup>29-31</sup> The in-line/online acquisition of analytical data allows to monitor at regular interval the reaction composition including hazardous or air sensitive intermediates with the possibility of adapting reaction

parameters or interrupt the flow process when a chemical or technical failure is detected (Scheme 1).



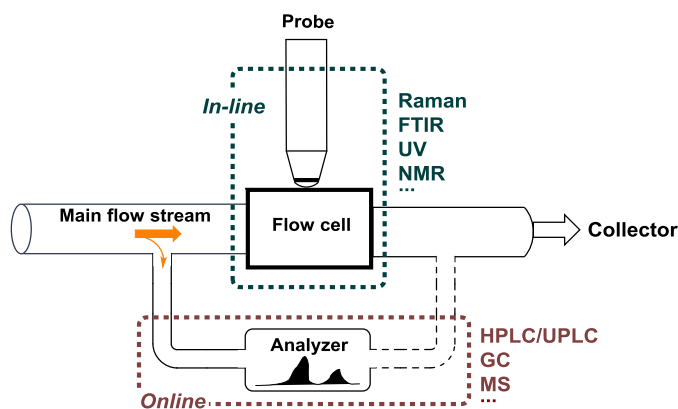
**Scheme 1.** Flow reactor integrating an in-line/online analytical tool

This review aims at presenting the main in-line/online analytical tools which can be integrated into flow reactors for the monitoring of chemical reactions. One frequent question arising with the integration of an analytical tool in a flow reactor for the monitoring of a chemical transformation is: “what is the most suited analytical solution to monitor my reaction?”. The response being complex by nature because multi-factorial, chemists are often plunged into a time-consuming hesitation, particularly when the decision to use a particular analytical tool involves purchasing the corresponding (expensive) machine. Therefore, rather than reporting an exhaustive compilation of publications describing the use of integrated in-line/online analytical tool to flow reactors, this review is more intended to serve as a guide for synthetic chemists. Analytical tools operating off-line and not physically connected to the flow reactor are not considered through this review as they require manual handling. This contribution reflects our personal view based on our own experience and is illustrated by selected published examples from leading research laboratories.

### **In-line versus online analysis**

Before disclosing the most relevant integrated analytical solutions in the arsenal of synthetic chemists, a complete understanding of the difference between in-line and online analysis is a prerequisite for a proper selection of equipment (Scheme 2). An in-line analyzer is equipped with a probe or a flow cell inserted or connected, respectively, to the main flow stream. It is flowed by the totality of the sample and it does not modify nor the concentration neither the flow rate. On the other hand, an online analyzer is installed on a sub-line and

connected to the main stream through a by-pass. It only receives a fraction of the sample diverted in the sub-line through a valve. The sample can be either redirected to the main stream after analysis or sent to the trash depending on the destructive nature or not of the analyzer.



**Scheme 2.** In-line versus online analyzer setups.

### **Real-time *versus* non-real-time analysis**

Analytical solutions can be roughly classified into two categories: those allowing real-time analysis and those requiring a certain period of time to collect analytical data. Interestingly, there is no universal definition for “real-time” as the physical time represented by this term mainly depends on the scientific area considered. In chemical synthesis, analyses are considered to be real-time when the time required for the entire analysis procedure (sampling time, data acquisition and data processing) is sufficiently fast compared to the chemical evolution of the reaction media. Fourier-transform infrared spectroscopy (FTIR), ultraviolet-visible (UV-Vis) and Raman spectroscopies are commonly considered as real-time analytical tools as they provide spectra in a matter of seconds. On the other hand, non-real-time analytical solutions mainly include chromatographic analysis techniques such as gas chromatography (GC) and high/ultra-performance liquid chromatography (HPLC/UHPLC). With such analytical solutions, several minutes are required to obtain a chromatogram and additional sample preparation may be required. NMR spectroscopy is a singular case which can be considered as a real-time tool for 1-dimensional  $^1\text{H}$  or  $^{19}\text{F}$  experiments and 2-dimensional experiments using ultra-fast pulse sequences. On the other hand, standard  $^{13}\text{C}$  experiments and 2D NMR sequences require at least several minutes in the most favorable cases and cannot be used for real-time monitoring.

## ■ DISCUSSIONS

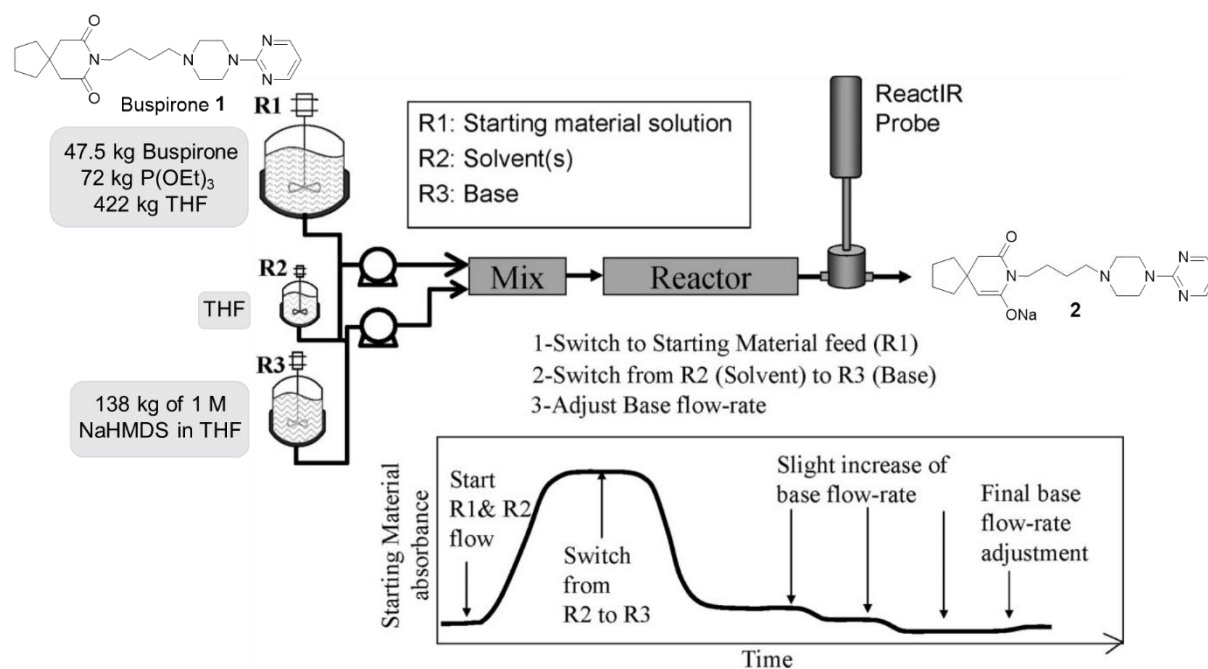
### Real-time analysis

**FTIR spectroscopy.** Infrared spectroscopy is likely the most frequently integrated real-time analytical tool in flow reactor. Infrared spectroscopy exploits the capacity of molecules to vibrate, through the bending or stretching of chemical bonds, upon absorption, emission or reflection of infrared radiations. The infrared region is divided into the near, mid and far-infrared, but for most applications only the mid-region, at wavenumbers ranging from 4000 to 400  $\text{cm}^{-1}$ , is useful in describing fundamental vibrations. As the vibrational energy levels are structure specific, each molecule produces a unique pattern in the ‘fingerprint’ region from 1500 to 500  $\text{cm}^{-1}$ . While this region might be, in theory, used for structure identification, the high number of overlapped bands complicates spectral analysis, especially in the case of mixture of compounds. This issue is the main reason for which infrared spectroscopy is much more useful, in routine, for observing the formation and the disappearance of functional groups in small organic molecules, essentially at wavenumbers  $>1500 \text{ cm}^{-1}$ .

Typical infrared spectrometer commercialized for integration into a flow reactor for in-line analysis consists of a FTIR spectrometer connected to a microscale flow cell integrating an attenuated total reflectance (ATR) silicon or diamond sensor. Silicon sensors are typically compatible at pH ranging from 1 to 10 and are transparent to wavenumber ranging from 4000 to 650  $\text{cm}^{-1}$ . Diamond sensors are more robust at highly basic pH but are not transparent between 2000 and 2250  $\text{cm}^{-1}$ , an important region to observe the stretching bonds of azides, isocyanate, thiocyanates, carbodiimides and disubstituted alkynes. ATR-FTIR spectroscopy is a nondestructive analytical tool particularly well suited to monitor the changing chemical composition of a sample without needing to interrupt the flowing, to modify reaction conditions and to handle sample preparation.<sup>32</sup> The high robustness of the probes toward either acidic or basic conditions in a large range of temperatures from -80 °C to 60 °C and at pressure up to 35 bar, allows in-situ analyses in a large variety of experimental conditions including the use of strong acids and bases.

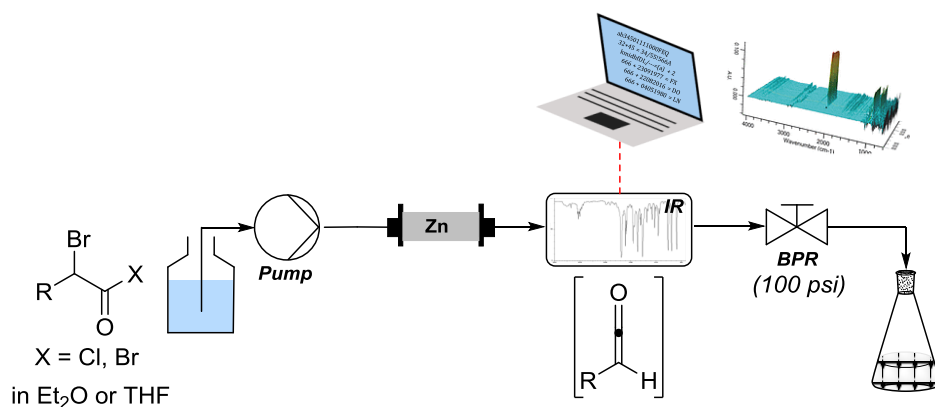
In this frame, LaPorte and co-workers reported the use of flow FTIR spectroscopy to monitor the enolization of buspirone **1** by deprotonation with NaHMDS (Scheme 3).<sup>33</sup> In order to identify the proper stoichiometry of NaHMDS, they started at a flow rate of the base feed slightly lower than estimated. The flow rate of the base feed was then progressively

increased at 1% increments until the total disappearance of the buspirone signal which corresponded to the complete conversion of buspirone **1** to the corresponding enolate **2**.



**Scheme 3.** Continuous flow reactor setup with integrated ATR-FTIR analysis. Visualization of the FTIR monitoring as the inset. Reprinted with permission from ref. 33. Copyright 2008, American Chemical Society.

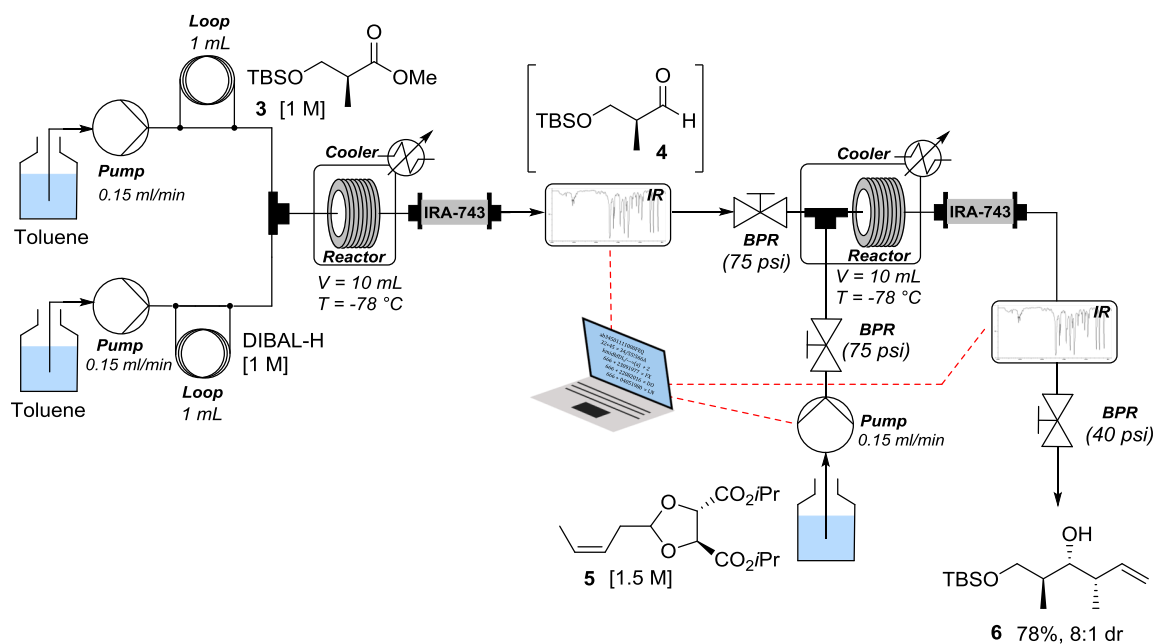
The ability of FTIR spectroscopy to deliver analytical data in quasi-real-time allows to observe the formation of unstable<sup>34</sup> or hazardous<sup>35</sup> intermediates that would be otherwise difficult to characterize by off-line analysis. Ley and co-workers described the monitoring of highly reactive monoalkyl and phenyl ketenes prepared in flow by a dehalogenation procedure (Scheme 4).<sup>34</sup> Ketenes can be ideally monitored by FTIR spectroscopy by their characteristic band at *ca.* 2100 cm<sup>-1</sup> using an ATR silicon sensor. The flow setup consisted of a single stream flowing a solution of  $\alpha$ -bromoacyl bromides in THF or Et<sub>2</sub>O and a glass cartridge loaded with zinc dust where the dehalogenation occurred. The FTIR spectrometer connected to the cartridge outlet monitored the band at around 2100 cm<sup>-1</sup> and provided important information. For instance, it was determined that the concentration of  $\alpha$ -bromoacyl bromides in Et<sub>2</sub>O had to be in the range of 0.05 to 0.1 M since more concentrated solution led to the formation of side-products, easily detected by FTIR. Moreover a continuous recording of FTIR spectra of ketene solutions over the time showed that the formed ketene had to be quickly reacted in the next step since it degraded after 30 minutes.



**Scheme 4.** Continuous flow reactor setup with integrated ATR-FTIR analysis for the synthesis of ketenes. The FTIR spectra were adapted with permission from ref. 34. Copyright 2015, Georg Thieme Verlag KG Stuttgart.

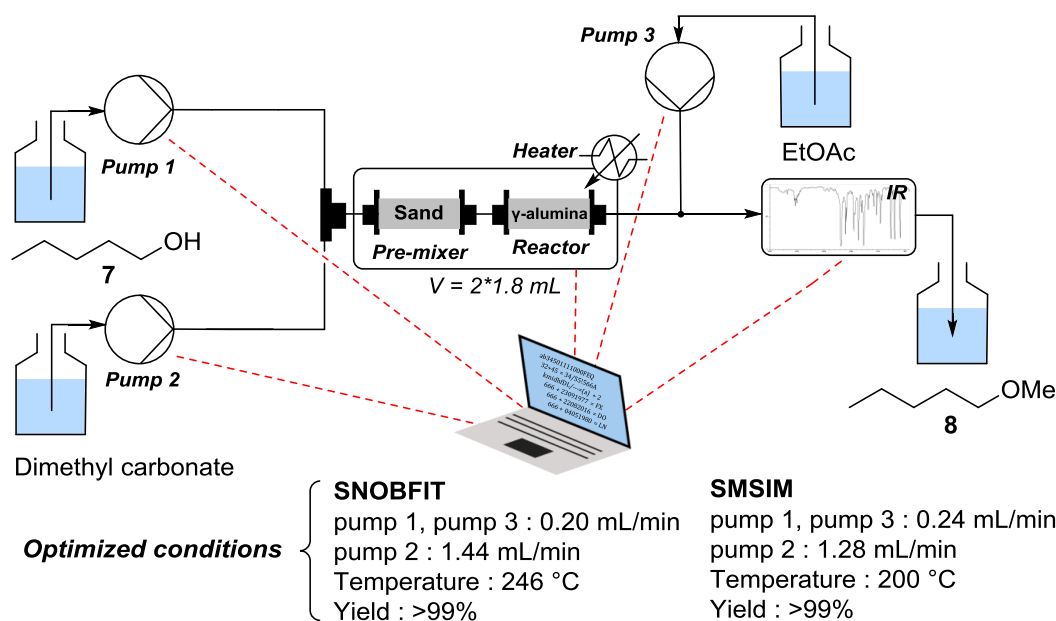
The absence of daily maintenance of FTIR spectrometer is a clear benefit in continuous manufacturing, since transformations can be continuously followed over several hours or even days.<sup>36</sup> FTIR signals have also been used to either adapt experimental conditions or trigger actions in real-time, for instance with the additional introduction of reagents in multi-step flow sequences.<sup>37-38</sup> Ley *et al.* described a two-step ester reduction-Roush crotylation sequence using a three stream flow platform (Scheme 5).<sup>39</sup> A FTIR spectrometer was placed immediately after the reactor coil in which ester **3** was reduced to the corresponding aldehyde **4** with DIBAL-H such as the third stream, flowing the Roush crotyl reagent **5**, could be automatically switched on upon detection of the aldehyde signal at  $1737\text{ cm}^{-1}$ . In order to facilitate the monitoring, two columns of IRA-743 resin were used to scavenge both the aluminum and boron residues. A second FTIR spectrometer monitored the formation of the expected product **6**. Benefit resulting from the use of this integrated flow platform is obvious since the entire flow process was completed in only three hours, in 78% yield, while a full working day was required to perform the two individual transformations through a traditional batch process.





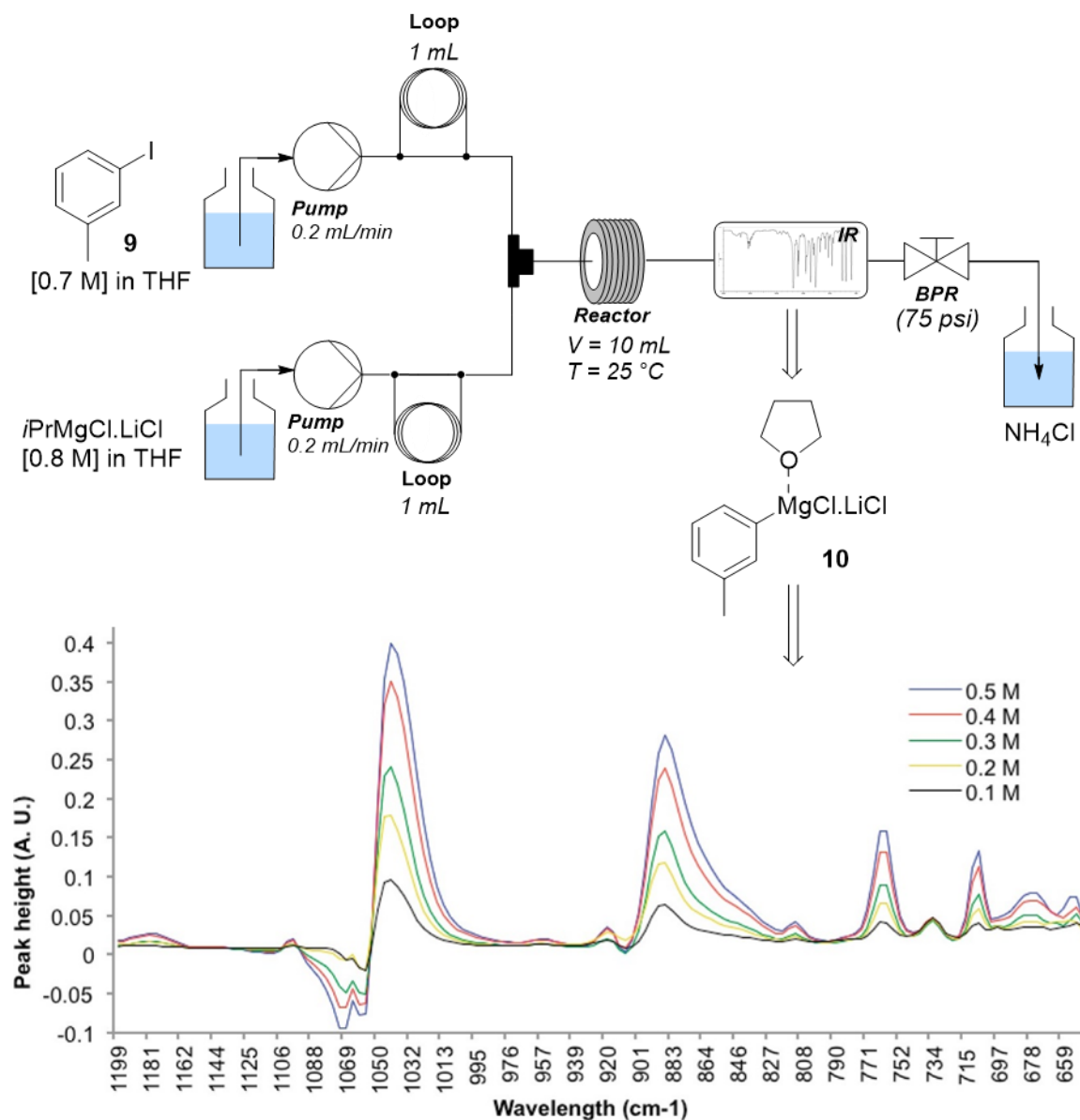
**Scheme 5.** Continuous flow reactor setup with two integrated ATR-FTIR spectrometers for the two-step ester reduction-Roush crotylation sequence.<sup>39</sup>

Combining an automated flow reactor with an in-line FTIR analysis and a feedback optimization algorithm resulted in a self-optimizing system.<sup>40-43</sup> Such powerful autonomous unit is able to perform optimizations with minimal user intervention after initialization. A self-optimizing flow platform consists of devices and instruments including the in-line/online analytical tool connected through communication ports (Ethernet, RS-232...) to a computer running a process-control software (LabVIEW, LabVision, ad-hoc software...) and an optimization algorithm. Poliakoff, Bourne and co-workers optimized the methylation of 1-pentanol **7** with dimethyl carbonate (DMC) under supercritical carbon dioxide (scCO<sub>2</sub>).<sup>44</sup> The flow setup consisted of two pumps fed with 1-pentanol **7** and DMC, respectively (Scheme 6). The two streams met in a mixer and the reaction occurred in a cartridge filled with Puralox NWA-155a  $\gamma$ -alumina powder as the catalyst. A third pump, downstream of the reactor, added ethyl acetate as the internal standard. The crude mixture was analyzed on the fly by a FTIR spectrometer connected at the reactor outlet and the resulting yield, calculated by monitoring the bands at 850 cm<sup>-1</sup> (ethyl acetate) and 1120 cm<sup>-1</sup> (pentyl methyl ether **8**) was sent to an optimization algorithm (either SNOBFIT or SMSIM) which proposed a new set of experimental conditions. The optimization was carried out in a 3-dimension space using the flow rate of each stream and the temperature as continuous variables. The speed of the FTIR analysis is a decisive advantage to proceed optimizations requiring a high number of experimental points in a reasonable time.



**Scheme 6.** Self-optimizing flow reactor with integrated ATR-FTIR spectrometer for the synthesis of pentyl methyl ether **8**. Dashed red lines refers to computer-controlled units.<sup>44</sup>

While the qualitative monitoring of bands of interest is convenient to follow the changing composition of a reaction mixture, the determination of yield, concentration or kinetics require a careful quantitative analysis. Quantification by FTIR spectroscopy is possible through the determination of either the area or the height of the bands of interest. The complexity of IR spectra, especially in the case of reaction mixtures, requires to carefully select the bands to be monitored and eventually use peak deconvolution and solvent subtraction methods. Knochel, Ley and co-workers monitored the formation and determined the concentration of aryl Grignard reagents using quantitative ATR-FTIR spectroscopy (Scheme 7).<sup>45</sup> Aryl Grignard reagent **10** were prepared by Mg/halogen exchange using a two-stream flow setup integrating a FTIR spectrometer connected to the flow reactor outlet. Despite many band overlaps, the authors were able to calculate the concentration of aryl Grignard reagents using the intensity of the band at  $767\text{ cm}^{-1}$  in the fingerprint area.



**Scheme 7.** (a) Continuous flow reactor setup with integrated ATR-FTIR spectrometer for the Mg/I exchange reaction. (b) Intensity of IR peaks of Grignard reagent **10** at different concentrations. IR spectra were reproduced with permission from ref. 45. Copyright 2012, American Chemical Society.

The high speed data acquisition provided by FTIR spectroscopy allows the determination of kinetic data. In this frame, Röder and co-workers elaborated an integrated microreactor with a FTIR spectrometer to collect kinetic data related to the deprotonation of a CH-acidic aromatic compound with *n*-butyllithium at temperature ranging from -35 to -15 °C.<sup>46-47</sup> By the means of several kinetic approaches, the authors concluded that the deprotonation occurred through the formation of *n*-butyllithium aggregates in THF to give a highly reactive and nonisolable lithiated product.

The detection sensitivity of FTIR spectroscopy is a matter of debate deserving a clarification. The intensity of traditional transmission infrared spectroscopy mainly depends of both the path length of the cell and the nature of the sample analyzed. With long path length cells and/or samples in strongly absorbing media, the energy reaching the infrared detector is weak. This issue generally precludes the use of the transmission mode for the in-situ monitoring of chemical reactions. By contrast, with ATR infrared spectroscopy the intensity of the spectrum is not correlated to the sample thickness as it mainly depends of the concentration and absorptivity of the sample, the number of internal reflections within the sensor and the penetration depth of the IR light. As a consequence, ATR-FTIR is well suited for in-situ analysis of samples in strongly absorbing media and detection at the submillimolar scale is possible provided that the monitoring is carried out on bands with high extinction coefficient.

**Raman spectroscopy.** There are analogies between Infrared and Raman spectroscopies since both techniques are based on the interaction of light with matter, resulting in the change in vibration modes of molecules. However, the similarities end there, as Raman and IR spectroscopies differ in many ways and are more complementary than similar. While IR spectroscopy relies on the absorption, emission or reflection of light by molecules having dipole moments, Raman spectroscopy relies on light scattering by molecules resulting in a change of the polarizability. Raman spectrometers use a laser light source operating in the visible or near IR infrared region of the electromagnetic spectrum. The Raman effect is weak and the signal intensity is inversely proportional to the fourth power of the scattered light wavelength. Working with laser emitting at shorter wavelengths increases the Raman scattering and the signal-to-noise ratio. As a consequence, many Raman spectrometers uses visible laser in the blue and green domains. However, excitation wavelengths in the range of 400-550 nm are not widely suitable for the analysis of organic compounds since they can cause sample fluorescence whose signal overlaps with the Raman scattering peaks of interest. For instance, in a complex gas-liquid-solid system established for the oxidation of benzyl alcohol on Au-Pd/TiO<sub>2</sub> catalyst using micropacked-bed reactors, Gavriilidis *et al.* observed that fluctuations associated with the dynamic movement of gas and liquid within the catalyst bed led to different types of gas-liquid-solid interactions.<sup>48</sup> It was observed that when the Au-Pd/TiO<sub>2</sub> catalyst was not sufficiently wetted by the liquid phase, a strong fluorescence signal, attributed to the catalyst support, caused saturation of the detector and precluded any analysis. Therefore, Raman spectrometers integrated to flow reactors for chemical synthesis

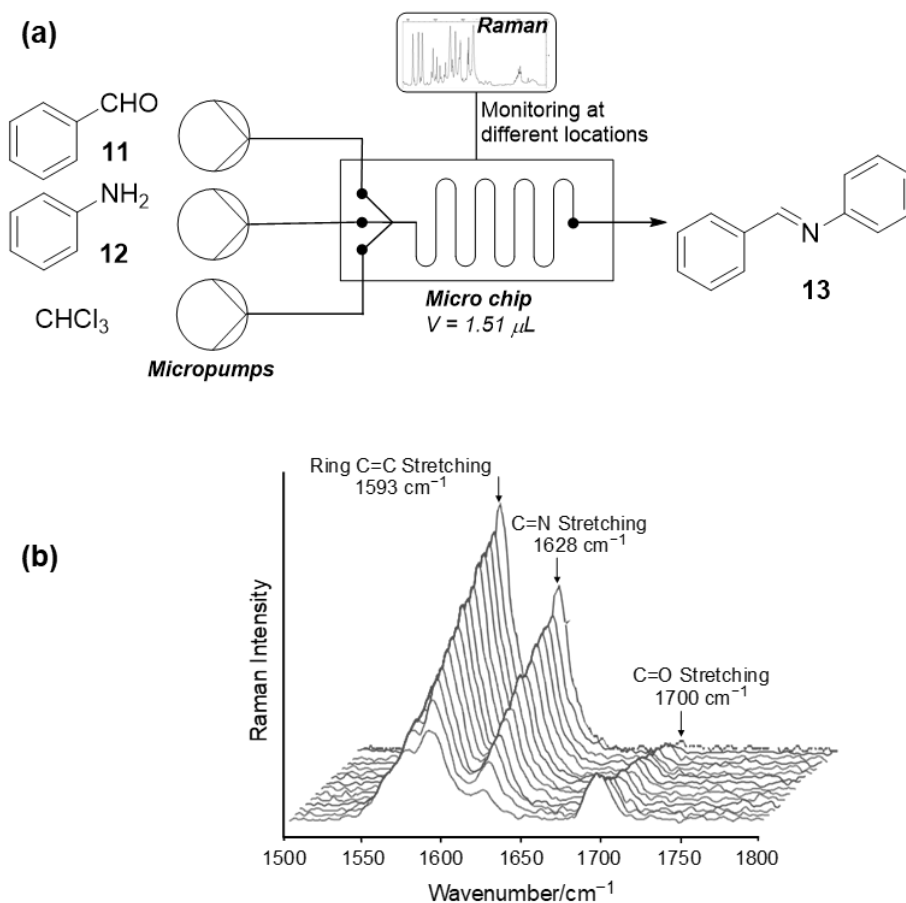
preferentially use 785 nm lasers which not only largely limits the sample fluorescence, but also preserves light sensitive compounds from degradation or rearrangement pathways. While pioneering studies regarding the monitoring of organic reactions by Raman spectroscopy used confocal Raman microscope systems,<sup>49</sup> the recent commercialization of benchtop systems equipped with a flow cell, greatly facilitated their integration in flow setups. Another important feature associated with the use of mid-IR laser is that most common materials of chip-type microreactors, including glass, quartz and polymeric materials, are transparent at 785 nm allowing the monitoring of reaction mixtures without immersion of the probe into the, sometimes corrosive, media.<sup>49</sup>

Raman spectroscopy belongs to the limited category of non-invasive and real-time spectroscopic techniques which can provide qualitative and quantitative structure information. Mansfield *et al.* collected quantitative data related to the rates and yields of the Pd/SiO<sub>2</sub>-catalyzed Suzuki cross-coupling reaction of aryl boronic acids with bromoarenes.<sup>50</sup> A custom built PTFE in-line flow cell equipped with a quartz window and coupled to a Raman probe allowed to monitor the chemical transformation in real-time. Associated to a calibration model, established from multivariate spectral data in the range of 1530 cm<sup>-1</sup> to 1640 cm<sup>-1</sup> using partial least-square regression, the in-line Raman monitoring enabled the determination of the second-order rate constant and the optimization of both the product conversion and the reaction yield.

Raman spectroscopy is also particularly useful for real-time in-line analysis of reactions carried out in alcohols or water for which FTIR spectroscopy can be problematic due to the strong absorption of mid-IR radiations by O-H bonds. The Raman scattering effect for hydroxyl bonds is weak and reactions carried out in alcohols and aqueous media can be, therefore, efficiently monitored by Raman spectroscopy. For instance, a microreactor coupled to an in-line Raman spectrometer was used to collect kinetic information regarding the Michael addition of aqueous piperidine on ethylacrylate.<sup>51</sup> The fast acquisition rate offered by Raman spectroscopy allowed to measure two hundred data points at different locations in the reactor channel in less than 1 hour.

The use of Raman spectroscopy is also particularly pertinent to identify functional groups with weak dipole moments which are difficult to characterize by IR spectroscopy, e.g., N=N, C=N, S-H, C=S...<sup>50</sup> In this line, Choo *et al.* followed imine formation by Raman microscopy from the condensation of benzaldehyde **11** with aniline **12** in a glass microfluidic chip with channel dimensions of 400 μm in width and 20 μm in depth (Scheme 8).<sup>52</sup> The disappearance of the C=O vibration band of benzaldehyde **11** at 1700 cm<sup>-1</sup> concomitantly to the appearance

of the intense C=N stretching band of imine **13** at  $1628\text{ cm}^{-1}$  were monitored by the confocal microscope at nine different points along the micro channel of the glass microchip to follow the reaction conversion.



**Scheme 8.** (a) Flow microreactor setup with integrated Raman spectrometer to monitor at different positions of the micro channel the imine formation. (b) Time dependent Raman spectra of imine formation at  $23\text{ }^{\circ}\text{C}$ . Each spectrum was recorded at time interval of 4 minutes. Raman spectra are reproduced with permission from ref. 52. Copyright 2003 John Wiley & Sons, Ltd.

Despite these clear assets, Raman spectroscopy remains comparatively much less used than IR spectroscopy for in-line analysis in flow reactors. Essentially, three reasons explain the relative disaffection of Raman spectroscopy by synthetic chemists for the real-time monitoring of chemical reactions. First, the Raman effect is weak, requiring, comparatively, higher concentrations of samples than for the FTIR spectroscopy. The Raman scattering can be increased by tuning the laser excitation power, but eventually at the detriment of the sample integrity. Second, band assignments of Raman spectra also suffer from a lack of full

understanding and access to spectral libraries is limited. It is therefore not surprising to see that many papers refer to the use of Raman spectroscopy for conversion measurement<sup>53</sup> or kinetic data generation<sup>54</sup> through the monitoring of an intense band of the product of interest but without reference to structure characterization. Last but not least, the higher cost of Raman spectrometers with respect to other conventional process analytical technologies, prevents Raman spectroscopy to be widely adopted in chemical laboratories for routine real-time analysis.

**Ultraviolet-visible (UV-Vis) spectroscopy.** Ultraviolet-visible absorption spectroscopy exploits the ability of compounds to absorb light in the ultraviolet (100-400 nm) - visible (400-750 nm) region of the electromagnetic spectrum. As a consequence, these atoms and molecules undergo electron transitions from the ground state to excited states (absorption) or from the excited states to the ground state (absorption/emission - luminescence). The lower is the energy gap between the HOMO and the LUMO frontier orbitals of the molecules, the more easily are excited the electrons and the longer is the wavelength of light the molecules can absorb. A proportional relationship between the absorbance of a solution at a given wavelength and the concentration of the absorbing species has been stated by the Beer-Lambert law (Equation 1).

$$A = \epsilon lc$$

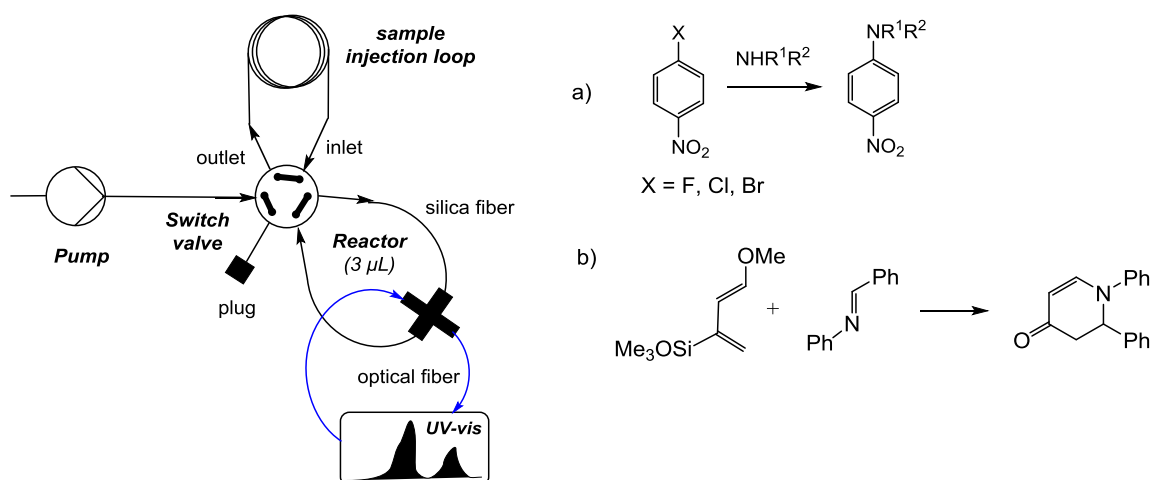
A = Absorbance  
 $\epsilon$  = molar attenuation coefficient  
l = optical path length in cm  
c = concentration of the attenuating species

**Equation 1.** Beer-Lambert law.

This correlation allows UV-Vis quantitative analysis of a sample through the response factor provided by an accurate calibration of the detector. The relevant modules of a UV-Vis spectrometer are the light source (deuterium or xenon arc lamps), a monochromator for splitting the different wavelength from the light, and a detector (photodiodes, photomultiplier tubes or charge-coupled devices CDD). Typically, the light is split into two beams inside the instrument: one is used as a reference and the other passes through the sample. The ratio of the two beams intensities is measured by the detector and the result is displayed as an absorbance (or emission if luminescence) vs wavelength graph. The peaks of this graph can identify (wavelength) and quantify (intensity) the composition of the sample. To avoid

absorption of optics and gases (160-180 nm), UV-Vis spectrometers are commonly set to handle high UV (190-390 nm) and Vis (390-770 nm) wavelengths where many organic and inorganic molecules absorb light through valence electrons of low excitation energy or charge-transfer absorptions respectively. An important advantage of UV-Vis spectroscopy is the rather low detection limit in the micromolar range compare to other analytical techniques. This high sensitivity comes at a price, since it requires to sample a fraction of the reacting mixture (online analysis) to avoid the saturation of the detector.

Online UV-Vis detectors have been coupled to microreactors to monitor the progress of a chemical transformation in terms of detection<sup>55</sup> or quantification<sup>56-57</sup> of analytes present in reaction mixtures. For example, Benito-Lopez *et al.*<sup>58</sup> applied online UV-Vis spectroscopy for the determination of chemical reaction kinetics in an very simple silica-based capillary microreactor integrating an UV-Vis analyzer through an optical-fiber connector. Nucleophilic aromatic substitutions and an aza Diels-Alder reactions were monitored online, for the first time under high pressure, at 391 nm and 335 nm respectively, without considerable safety precautions (Scheme 9).



**Scheme 9.** Continuous flow online UV-Vis monitoring of a) aromatic nucleophilic substitutions and b) aza-Diels-Alder reactions.<sup>58</sup>

More sophisticated silicon and quartz microreactors transparents to UV-Vis light (from  $\lambda = 325$  nm and 190 nm respectively) have also been employed in photochemistry.<sup>59</sup> In these examples, the light source and the detector were connected to optic-fibers placed in a region of the chip reactor adapted for optical measurements.

Despite these important examples, the use of online UV-Vis spectroscopy to monitor organic transformations conducted in continuous flow remains limited by the lack of



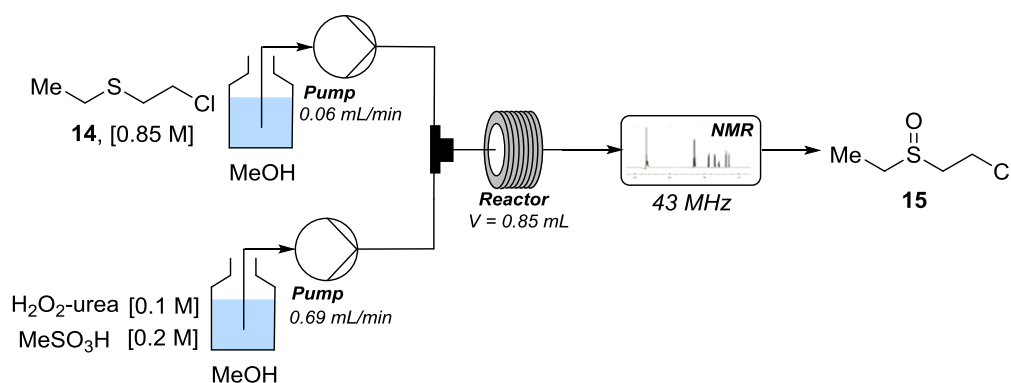
structural data and the need of working with UV-active compounds. It is therefore not surprising to see that most relevant examples of flow setup integrating UV-Vis spectroscopy were dedicated to inorganic transformations, essentially for the synthesis of metallic nanoparticles,<sup>60-66</sup> but the use of microfluidics in inorganic chemistry is out of the scope of this review.<sup>67</sup>

**Fluorescence spectroscopy.** Another optical spectroscopy implemented for online reaction monitoring has been the fluorescence spectroscopy<sup>68</sup> characterized by a very high sensitivity with detection limits in the nanomolar/picomolar range.<sup>69</sup> In this case, the emission light of fluorescent substances from the excited state, at a specific excitation wavelength, is detected and measured. Spectrometers consist of an exciting source (laser, light emitting diodes LED or lamps) and a detector (CDD microscope for example) as the most relevant items. These optical components have been integrated in chip microreactors through embedded optical fibers or on-chip waveguides. Most important applications of online fluorescence detection in flow processes involve the synthesis of inorganic nanoparticles<sup>57, 70</sup> and applications to organic transformations remains mostly overlooked,<sup>71</sup> likely because small organic molecules are rarely characterized by their fluorescence properties and the monitoring of non-fluorescent analytes would require an extra labelling step.

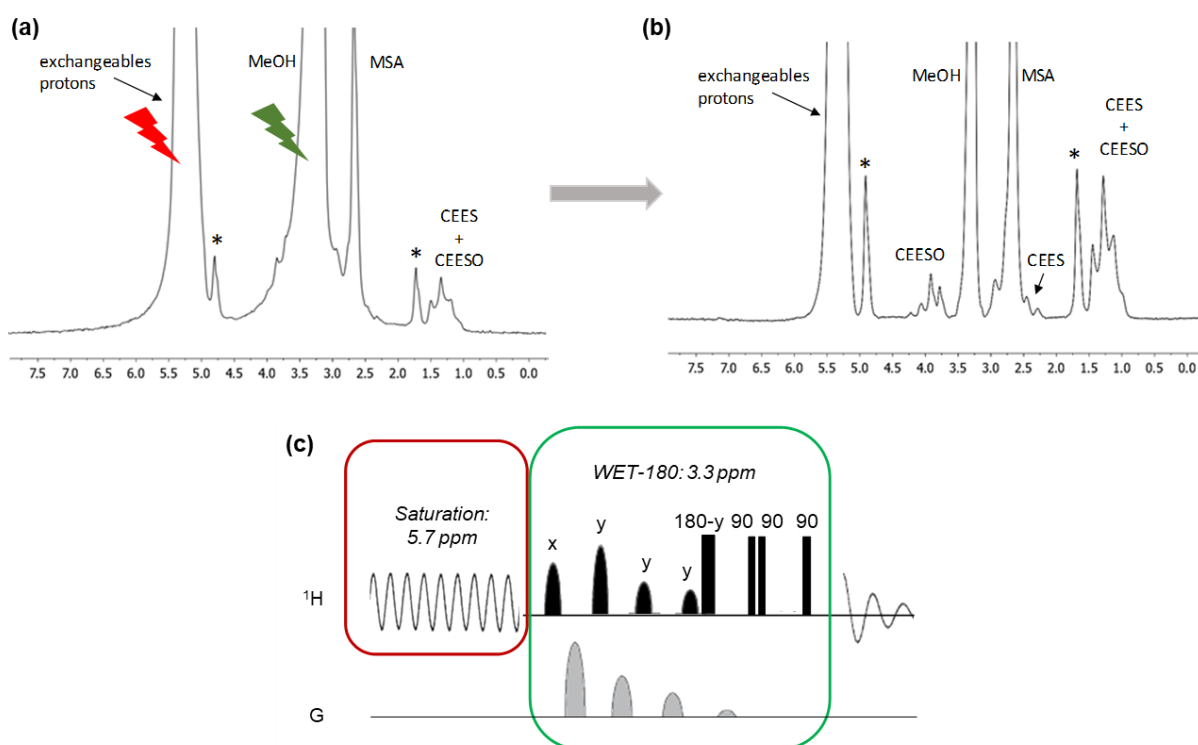
**NMR spectroscopy.** Benchtop nuclear magnetic resonance (NMR) spectroscopy is the most recent real-time analytical tool in the portfolio of synthetic chemists for in-line analysis under a fume hood. While high-field NMR spectroscopy is certainly the most powerful and universal spectroscopic technique at the disposal of organic chemists, it is poorly suited for monitoring chemical reactions in real time. The high cost of high-field NMR spectrometers, the necessity to have a specific equipment to connect the reactor (batch or flow) to the NMR tube and their location in dedicated areas, often far away from synthetic laboratories, prevent their use as in-line analytical tool on a routine basis. A new era appeared with the recent development and commercialization of a new generation of benchtop low- or medium-field NMR spectrometers equipped with a permanent magnet and working at frequencies ranging from *ca.* 40 to 100 MHz for the proton nucleus.<sup>72</sup> Benchtop NMR spectrometers are sufficiently compact to be placed under a fume hood, benefit from a reasonable cost (50-100 k€ *versus* >500 k€ for high field spectrometers) and their running cost are negligible since they do not require cryogenic fluids. Most commercially available benchtop spectrometers can detect at least <sup>1</sup>H, <sup>19</sup>F, <sup>13</sup>C, and <sup>31</sup>P nuclei but other nuclei such as <sup>29</sup>Si, <sup>7</sup>Li, <sup>15</sup>N, <sup>11</sup>B and

$^{23}\text{Na}$  can be detected as well with the most recent spectrometers. The miniaturization of NMR spectrometers inevitably comes at a price. The NMR sensitivity being proportional to  $B_0^{3/2}$ , where  $B_0$  is the static magnetic field, benchtop spectrometers are limited to the detection of relatively concentrated reaction mixtures, typically  $>50$  mM.

Benchtop spectrometers are particularly adapted to in-line analysis since they can record spectra of reaction mixture carried out in non-deuterated solvents. This obvious practical and economical asset also inevitably complicates spectral analysis due to the (often large) signal(s) from the solvent(s) which frequently overlap with the peaks of interest. The recent development of advanced flow-compatible solvent-suppression pulse sequences addressed this issue.<sup>73</sup> For instance, in the context of the development of an on-board flow device with an integrated in-line benchtop NMR spectrometer for the neutralization of mustard-gas simulants, Legros and co-workers developed an advanced solvent suppression method to monitor the oxidation of 2-chloroethyl ethyl sulfide (CEES) **14** by a hydrogen-urea complex (Scheme 10).<sup>74</sup> Measurement of the reaction conversion on the untreated spectrum of the reaction mixture was plagued by the strong resonances from the solvent (MeOH) at 3.31 ppm and from exchangeable protons (MeOH, MeSO<sub>3</sub>H, and H<sub>2</sub>O) at 5.37 ppm (Figure 1a). The WET-180-NOESY pulse sequence associated to a continuous wave presaturation to suppress signals at 3.31 ppm and 5.37 ppm, respectively, allowed to follow the disappearance of the CEES triplet at *ca.* 2.50 ppm together with the appearance of the resonance at *ca.* 4.90 ppm, attributed to the 2-chloroethyl ethyl sulfoxide (CEESO) **15**, (Figure 1 b-c).



**Scheme 10.** Continuous flow reactor setup with integrated benchtop NMR spectrometer for oxidation of mustard gas simulants.<sup>74</sup> Adapted with permission from ref. 72. Copyright 2018 Royal Society of Chemistry.

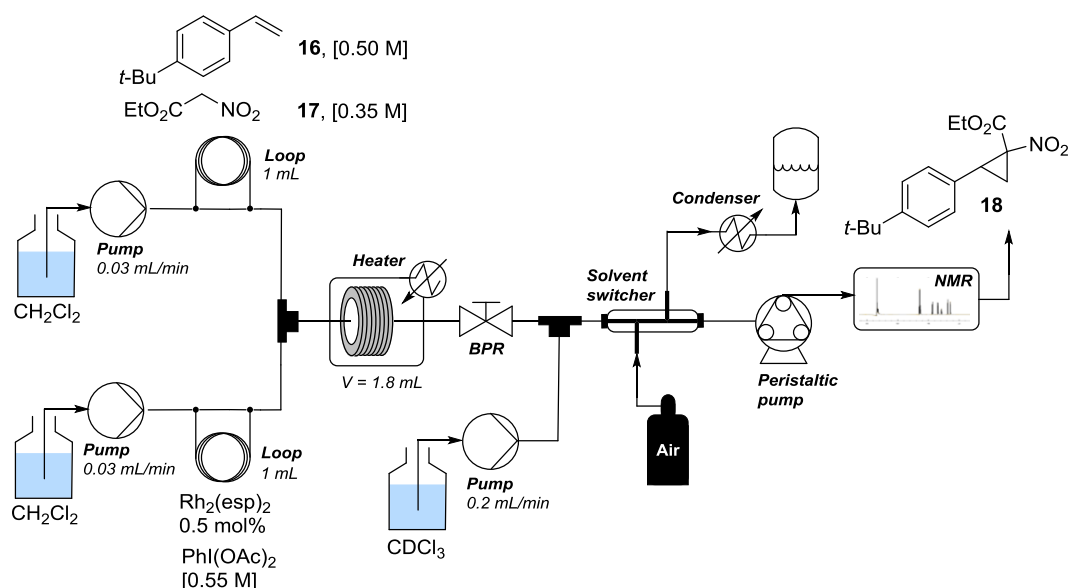


**Figure 1.** Low-field NMR spectra recorder under flow condition (a) without the solvent suppression method and (b) with the solvent suppression method. (c) WET-180-NOESY pulse sequence used to reduce the faraway solvent effect and to obtain a flatter baseline. Adapted with permission from ref. 74. Copyright 2017 Wiley- VCH Verlag GmbH & Co.

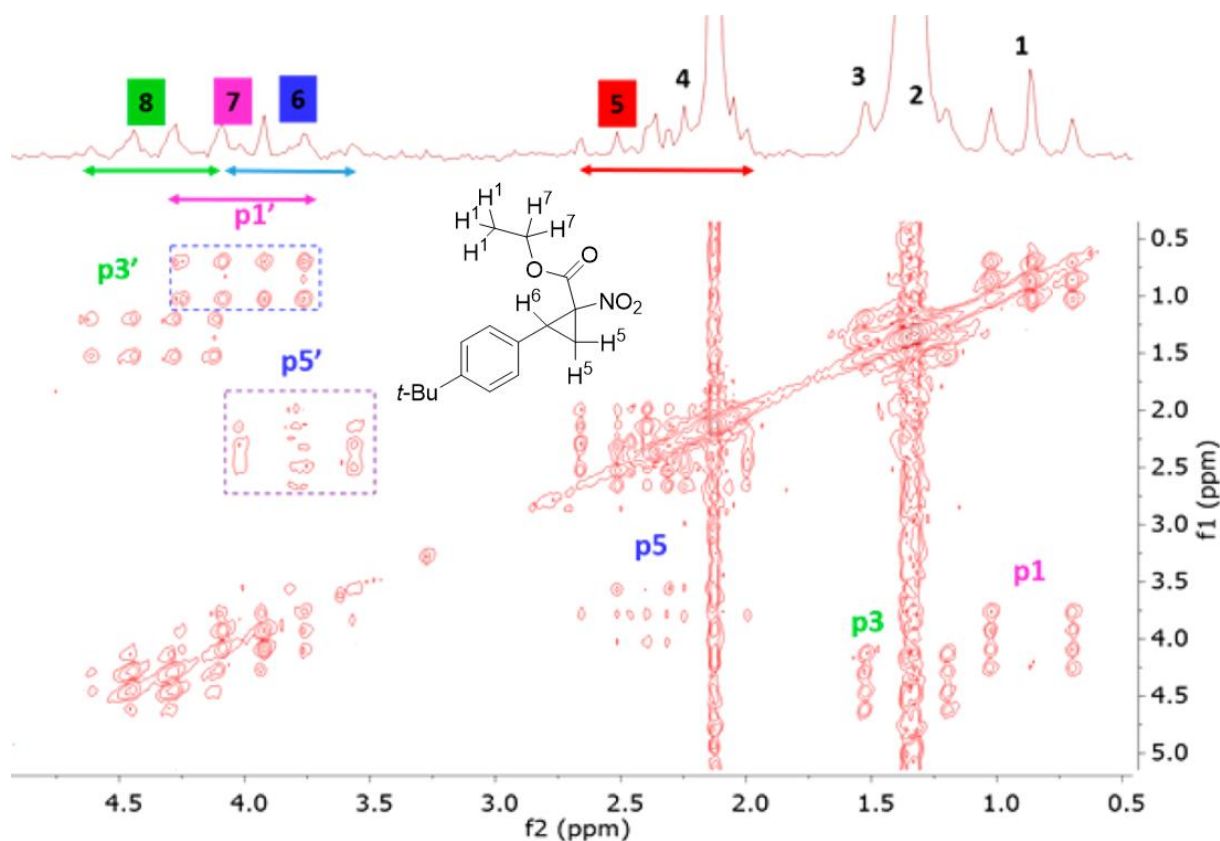
The group of Ley proposed an alternative to the use of solvent suppression methods with the invention of a simple solvent switching system, placed at the outlet of the flow reactor, to switch the protonated media used in the flow process for a deuterated solvent for the NMR analysis.<sup>75-76</sup> While this system proved to be particularly efficient to switch  $\text{CH}_2\text{Cl}_2$  for  $\text{CDCl}_3$  it might be limited with high boiling point solvent media.

The spectrometer resolution, *i.e.*, the ability to separate resonances, is also strongly impacted by the reduced magnetic field. For instance, for a typical 10 ppm spectrum, chemical shifts are spread over 600 Hz on a 60 MHz benchtop spectrometer and over 4000 Hz on a standard high-field 400 MHz spectrometer. In other words, the reduced frequency range of benchtop spectrometers increases the risk of signal overlap, especially with highly coupled molecules. The spectral resolution can be significantly enhanced by spreading resonances on a 2-dimensional (2D) map through the use of 2D experiments. While several homo- and heteronuclear 2D NMR pulse-sequences, including ultrafast methodologies, have already been implemented on benchtop spectrometers,<sup>77-78</sup> their use for online monitoring in a flow

reactor remains confidential. Ley and co-workers examined the potentialities of in-line 2D NMR experiments to monitor the continuous flow rhodium-catalyzed cyclopropanation of 4-*tert*-butylstyrene **16** with ethylnitroacetate **17** in CH<sub>2</sub>Cl<sub>2</sub>.<sup>76</sup> The 2-stream flow setup used to this end, also included a solvent switcher system placed at the outlet of the flow reactor to switch CH<sub>2</sub>Cl<sub>2</sub> for CDCl<sub>3</sub> and facilitate the spectral analysis without perturbing solvent peaks (Scheme 11). The authors developed a script to run a custom-made loop sequence of 1D <sup>1</sup>H NMR and 2D COSY, triggered every 13 minutes over 3 loops. This 2D pulse sequence allowed to monitor the starting materials consumption and the product formation even in overlapped area which were otherwise uninterpretable with standard 1D <sup>1</sup>H NMR (Figure 2). Depending on the reaction kinetics on the one hand and the required analysis time on the other hand, the use of 2D pulse sequences may or may not be considered as (quasi)real time.



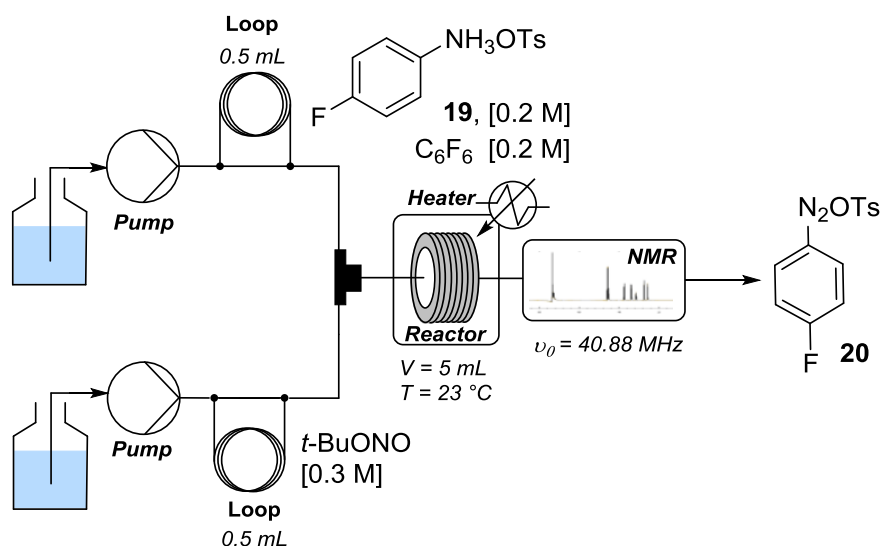
**Scheme 11.** Continuous flow setup with integrated benchtop NMR spectrometer for the cyclopropanation of 4-*tert*-butylstyrene **16** with ethylnitroacetate **17**.<sup>76</sup> Adapted with permission from ref. 72. Copyright 2018 Royal Society of Chemistry.



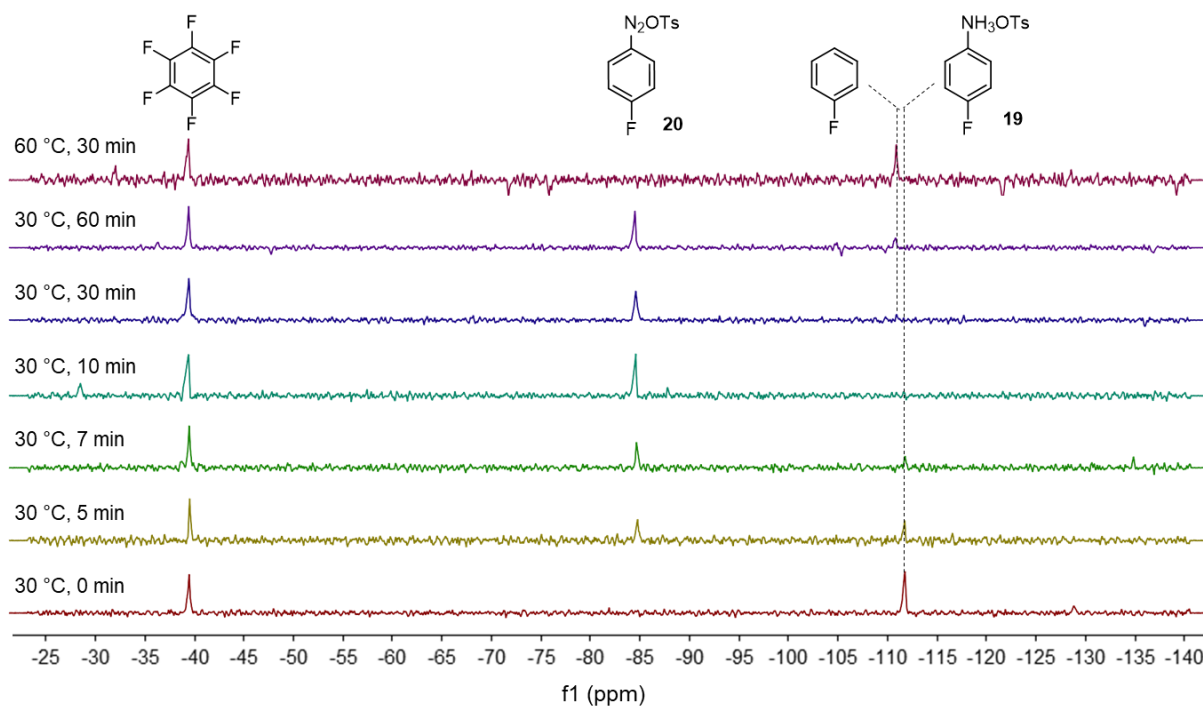
**Figure 2.** COSY spectrum at the steady-state between  $\delta_{\text{H}}$  0.5-5 ppm. Cyclopropane **18** was included for clarity. Protons 2 refer to *t*-Bu of **16** and **18**, protons 3 refer to  $\text{CH}_3$  of **17**, protons 4 refer to  $\text{PhI}(\text{OAc})_2$ , protons 8 refer to  $\text{CH}_2$  of **17**. The NMR spectrum was reproduced with permission from ref. 76. Copyright 2016 American Chemical Society.

The use of  $^{19}\text{F}$  experiments is a powerful alternative 2D pulse sequences, which can be time-consuming, when 1D  $^1\text{H}$  NMR spectra show inextricable signal overlaps, obviously provided that products being monitored bear fluorine atoms. The large range of chemical shift, from -300 to 150 ppm for most fluorinated organic compounds, significantly limit the risk of signal overlap. As an additional asset to the credit of  $^{19}\text{F}$  experiments, it must be mentioned that the  $^{19}\text{F}$  nucleus is 100% naturally abundant, allowing fast acquisition times with 83% of the  $^1\text{H}$  nucleus receptivity. Rehm *et al.* integrated a benchtop spectrometer into a continuous flow lab plant for the monitoring of three reactions, i.e., Krapcho decarboxylation, Ruppert-Prakash perfluoroalkylation and visible-light C-H arylation.<sup>79</sup> While rather different in nature and requiring different experimental setups, all these reactions successfully used fluorine atoms as a chemical probe for in-line  $^{19}\text{F}$  NMR analysis. Felpin *et al.* also exploited the asset of in-line monitoring by  $^{19}\text{F}$  NMR experiments for the continuous flow diazotization of fluorinated anilinium to arenediazonium salts.<sup>80</sup> Advantages of using in-line  $^{19}\text{F}$  NMR

analysis for the synthesis of arenediazonium salts is two-fold. First, the hazardous nature of arenediazonium salts prevent monitoring by standard methods such as off-line gas chromatography requiring additional sample handling. Second, by nature arenediazonium salts essentially bear aromatic protons and associated resonances often overlap with the resonances from the starting anilinium, limiting the use of  $^1\text{H}$  NMR. For instance, using a simple two-stream flow setup, the disappearance of 4-fluoroanilinium **19** together with the formation of the targeted 4-fluoroarene diazonium salt **20** could be easily followed by in-line  $^{19}\text{F}$  NMR analysis at 40.88 MHz with a pulse-sequence requiring *ca.* 60 seconds (Scheme 12). Interestingly, the in-line  $^{19}\text{F}$  NMR analysis allowed a strict control of the experimental conditions (30 °C, 10 min residence time) to maximize the conversion while minimizing the unwanted dediazotization pathway which occurred upon either extended residence time or increased reaction temperature (Figure 3).



**Scheme 12.** Continuous flow setup with integrated benchtop NMR spectrometer for the diazotization of anilinium **19** to 4-fluorobenzene diazonium tosylate **20**. Adapted with permission from ref. 80. Copyright 2021 Royal Society of Chemistry.



**Figure 3.** Monitoring of the diazotization of anilinium **19** to 4-fluorobenzene diazonium tosylate **20** in THF by in-line  $^{19}\text{F}$  NMR analysis at 40.88 MHz. Reproduced from ref. 80 with permission from The Royal Society of Chemistry.

**Mass spectrometry (MS).** Mass Spectrometry analyzers measure the mass-to-charge ratio of ionic fragments resulting from the ionization of a sample with a stream of electrons. The electrically charged particles generated by collisions in the ion source are separated according to their velocity or trajectory by the action of a static electric or magnetic field and quantitatively screened by a detector.

The choice of the ionization technique depends on the application. Hard ionization methods (*e.g.*, electron ionization) impart high quantities of energy to the sample, yielding a high degree of fragmentation and a highly detailed mass spectra. Soft ionization processes (*e.g.*, atmospheric pressure chemical ionization) provoke a little fragmentation of the sample since they deliver small amounts of energy to the sample. The most commonly used soft ionization techniques are fast atom bombardment (FAB), chemical ionization (CI), electrospray ionization (ESI) and matrix-assisted laser desorption/ionization (MALDI).

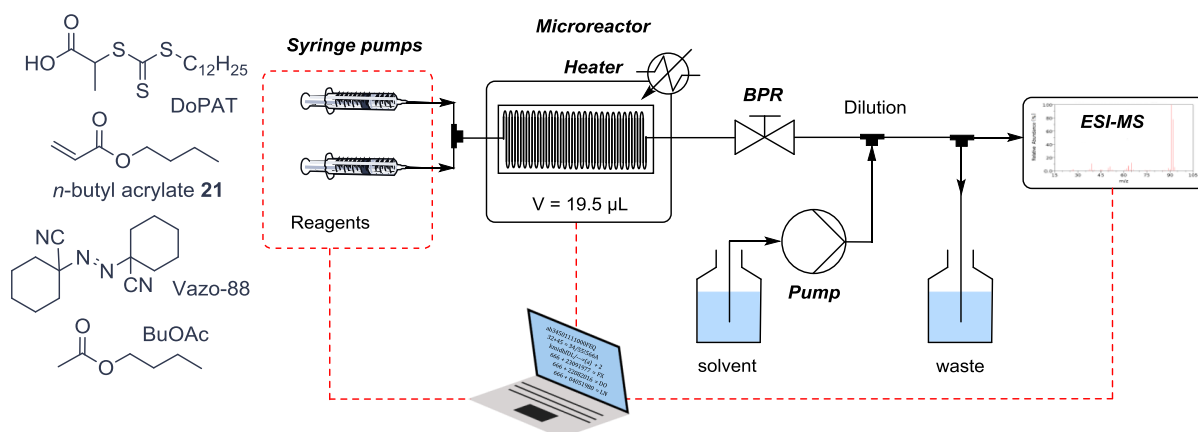
The most commonly used mass analyzers include time-of-flight (ToF), quadrupole, ion trap, orbitrap and magnetic sector analyzers. ToF analyzers separate ions with very good resolution according to their  $m/z$  ratio based on the time they need to reach the detector. Separately, ions entering a quadrupole analyzer have trajectories modified proportionally to their  $m/z$  values as a result of an alternating radio frequency delivered. Depending on the radio

frequency applied, only specific  $m/z$  values are detected. They are especially well-suited for interfacing chromatographic techniques as only low vacuum levels are required. Although they suffer from a poor resolution, good sensitivities characterize them. Ion trap analyzers use a similar principle than quadrupole ones but they are smaller, relatively cheap and present a good sensitivity and resolution. Besides, magnetic sector analyzers disperse ions by modifying their trajectories according to their  $m/z$  ratios under the effect of a magnetic field. These analyzers are characterized by high sensitivities and resolutions although they are expensive and require skilled users. Finally, in orbitrap analyzers an electric field spreads ions of a specific  $m/z$  value in several orbits inside the trap in a way that their frequency of rotation is directly related to their  $m/z$  value. These analyzers are characterized by a very high resolution and accuracy as well as a relatively small size. High resolution spectrometers providing the exact mass of isotopic species are commonly equipped with ToF, orbitrap or magnetic sector analyzers. They allow to differentiate between compounds with the same nominal mass and to determine elemental compositions. On the other hand, quadrupole and ion trap analyzers will be suitable for low resolution mass spectrometers.

Compared to other PAT, MS is characterized by higher resolution and sensitivities than benchtop NMR spectroscopy, and easier data representation than other spectroscopy techniques such as vibrational ones.

Online ESI-MS has been implemented to study mechanisms of organic and organometallic transformation in flow as well as to characterize reaction intermediates “on the fly” due to the high sensitivity of this technique and the controlled reaction conditions in continuous flow devices.<sup>81-84</sup> Online high resolution MS has been a notably useful PAT in giving access to quasi-continuous data acquisition through the use of a continuous flow microreactor connected to an Orbitrap mass spectrometer to monitor the RAFT polymerization of *n*-butyl acrylate **21** (Scheme 13).<sup>85</sup> The entire polymerization reaction was monitored in a single experiment allowing rapid kinetic screening, especially for the determination of polymer end groups, with the provided real time data. Online MS is especially well suited for the determination of specific end group patterns where other classical spectroscopic analytical techniques (NMR, IR, UV-Vis) are limited. High reliability was proven combining continuous flow with online MS analysis. This strategy afforded weakly dispersed data with high time resolution.





**Scheme 13.** Flow microreactor platform with integrated online ESI-MS analysis. Dashed red lines refers to computer-controlled units.<sup>85</sup>

In principle, quantification of a product is possible with MS detectors by measuring the area enclosed by its peak if adequately adjusted to the detector response factor.<sup>86</sup> However, crude reaction mixtures including several products represent a challenge as the ionization ability of each compound, and thus the intensity of their MS peaks, may be different, independently of their concentration. Moreover, when passing through the MS ion source, compounds may ionize differently whether they are alone or in the presence of other molecules, misrepresenting their concentration. Hence, ionized molecules that could be detected alone, may not be detected in a reaction mixture. This is the main reason for which the combination of chromatographic techniques with MS enables precise and reliable quantification (*vide infra*).

### Non real-time analysis

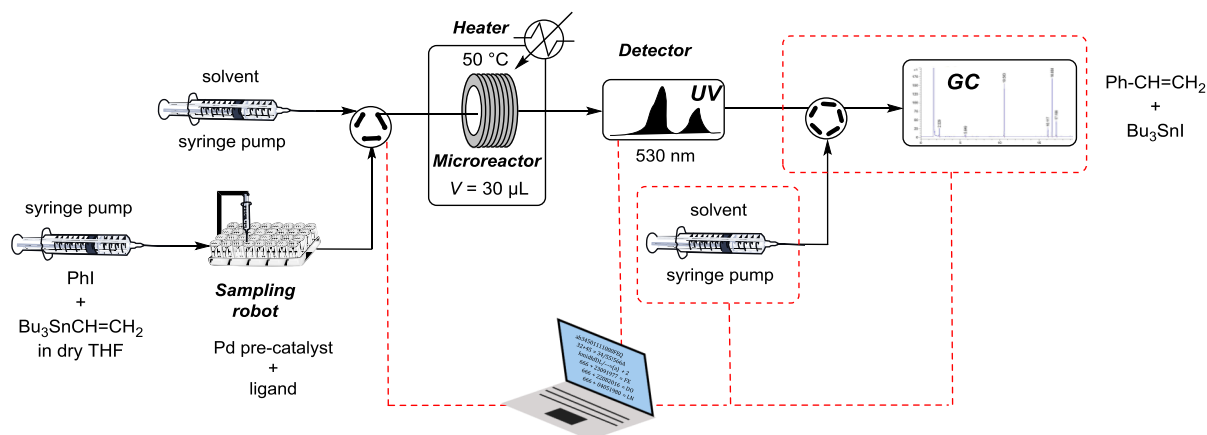
The integration of real-time analytical tools into flow reactors constitutes the finest solution to monitor and control chemical transformations. However, non-real time analytical devices can fulfill the criteria of an efficient flow process when real-time tools lack of universality in specific transformations and/or when the time required for the analysis is not significant compared to the time required for the global flow process.

Chromatographic techniques are usual and efficient analytical methods for the separation of substances from a chemical mixture and their quantitative analysis. The chemical separation operates between two phases, the mobile phase carries the mixture and moves through the stationary phase with whom the analytes interact and are and are separated according to their physical and chemical properties. An optimal separation is essential for

performing monitoring applications and should provide a chromatograph where each peak, characterised by a specific retention factor, correspond to a different compound. The specific chemical and physical properties of an analyte determines its retention factor which measures how long the chemical substance will be retained in the stationary phase before emerging from it with the mobile phase. Depending on the study case, gas chromatography (GC), liquid chromatography (LC) techniques and supercritical fluid chromatography (SFC) can be implement as a function of the nature of the mobile phase.

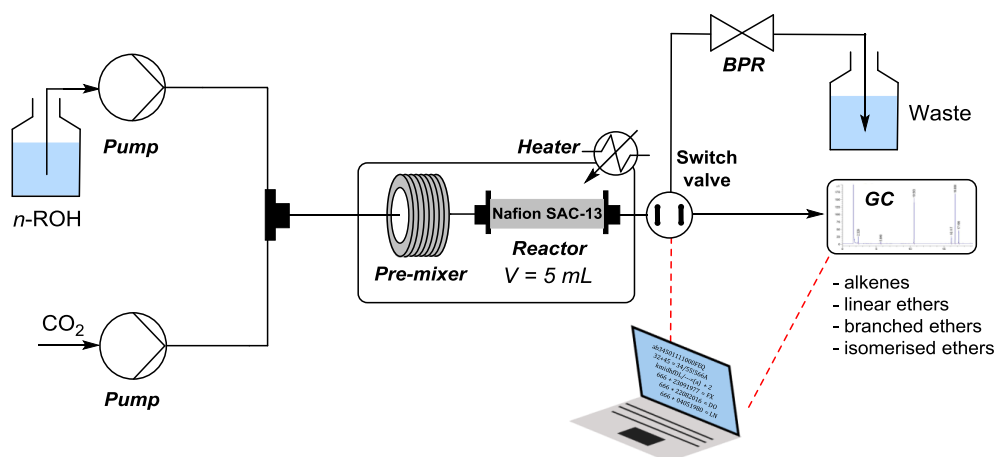
**Gas Chromatography (GC).** In gas chromatography, the sample (liquid or gas) is injected in an inert gas carrier (mobile phase) which flows through a heated (oven) stationary phase (solid support inserted in a column). Specific interactions (adsorption) take place between the stationary phase and the analytes. As a consequence, the analytes exit the column at different times depending on their chemical and physical properties and this separation is monitored by a detector such as a flame ionization detector (FID), commonly used for sample analysis.

As a chromatographic technique, online GC analysis cannot provide structural data. It is therefore more suited for the screening of experimental conditions leading to a known target which, by nature, does not require structure identification or elucidation. In this frame, online GC has been used by Weber and co-workers for catalyst discovery through a combinatorial approach.<sup>87</sup> They studied the Pd-catalysed Stille cross-coupling reaction in the presence of a variety of Pd-based precatalysts and phosphine or arsine ligands. To this end, online GC analysis was connected to a microliter capillary flow platform including syringe pumps, an autosampler, a fused-silica capillary microreactor, a heating device, an optical fiber UV-visible detector for finding the reaction zones, and a sample loop connected to the GC analyser (Scheme 14). Although reagents and products (reaction zones) were loaded, ejected and analysed serially, inside the reactor, these solvent zones reacted in parallel. The 30  $\mu\text{L}$  capillary microreactor could locate up to 15 reaction zones coexisting at 0.25  $\mu\text{L}/\text{min}$ . For this study, the entire procedure was carried out under inert atmosphere, including the GC analysis, preventing products from decomposition occurring in traditional offline work-up/analysis steps.



**Scheme 14.** Flow microreactor screening platform integrating online UV-Vis detection and online GC analysis. Dashed red lines refers to computer-controlled units.<sup>87</sup>

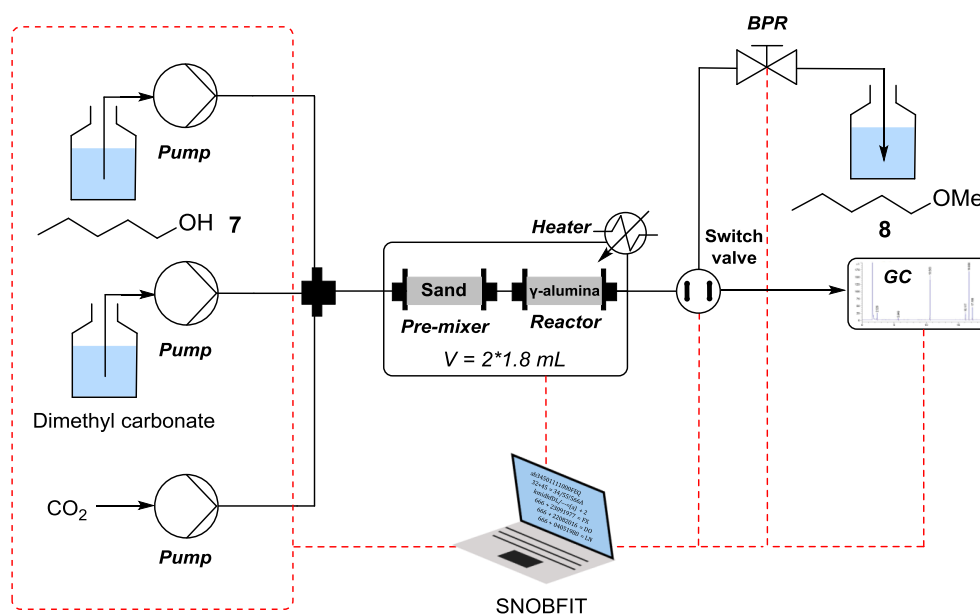
Being particularly well suited to study gaseous or volatile compounds,<sup>88</sup> GC has been integrated in an automated continuous flow platform by Poliakoff and co-workers to study the acid-catalysed etherification of short chain alcohols in supercritical CO<sub>2</sub>.<sup>89</sup> The flow setup included HPLC pumps, a mixer, a reactor (5 mL), a heating system, an electronic 4-port HPLC switch valve and an online isothermal GC device (Scheme 15). Commonly lost during the scCO<sub>2</sub> expansion step, volatile products could, in this way, be detected and analysed by direct GC sampling.



**Scheme 15.** Automated flow platform integrating online GC analysis for the study of the etherification of alcohols. Dashed red lines refers to computer-controlled units. Pumps, BPR and temperature are controlled by on-board processors.<sup>89</sup>

In a further improvement, this supercritical technology was also deployed with the use of a self-optimizing flow platform<sup>90-95</sup> to optimize the output of homogeneous and heterogeneous

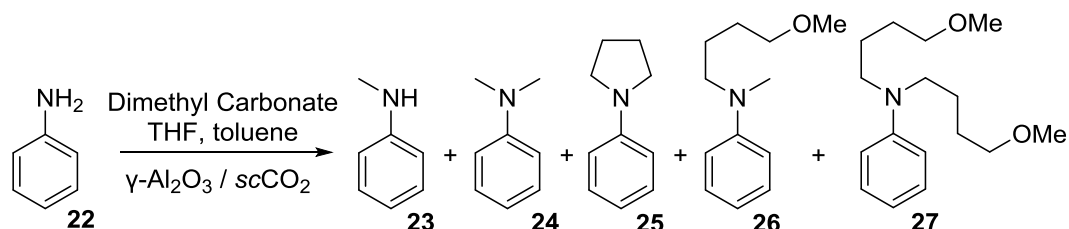
continuous flow catalyzed reactions such as hydrogenations, aldol condensations and methylations in supercritical fluids. Impressively, such self-optimizing device, integrating the SNOBFIT algorithm, enabled the optimization of the output composition for different products from the same etherification process, in only 35 h vs 83 days for an equivalent manual optimization campaign (Scheme 16).<sup>91</sup> Well suited for the analysis of highly volatile compounds, the GC was the monitoring device of choice. The online GC analysis allowed the evaluation of the sustainability of the studied reactions, from an environmental and an economic point of view, in terms of five different criteria (yield, productivity,  $E$  and  $E+$  factors and a crossed factor between the yield and the productivity). Typically, a control software was set to divert samples from the reactor flow towards the GC analyzer through a high-pressure sample loop. A non-polar capillary column was used considering the presence of alkyl chains in the products, and a standard temperature-ramp program lasting 17 min was set from 40 °C to 200 °C for an efficient separation of the analytes.



**Scheme 16.** Self-optimisation flow platform integrating online GC for the methylation of alcohols with DMC in  $scCO_2$ . Dashed red lines refers to computer-controlled units.<sup>91</sup>

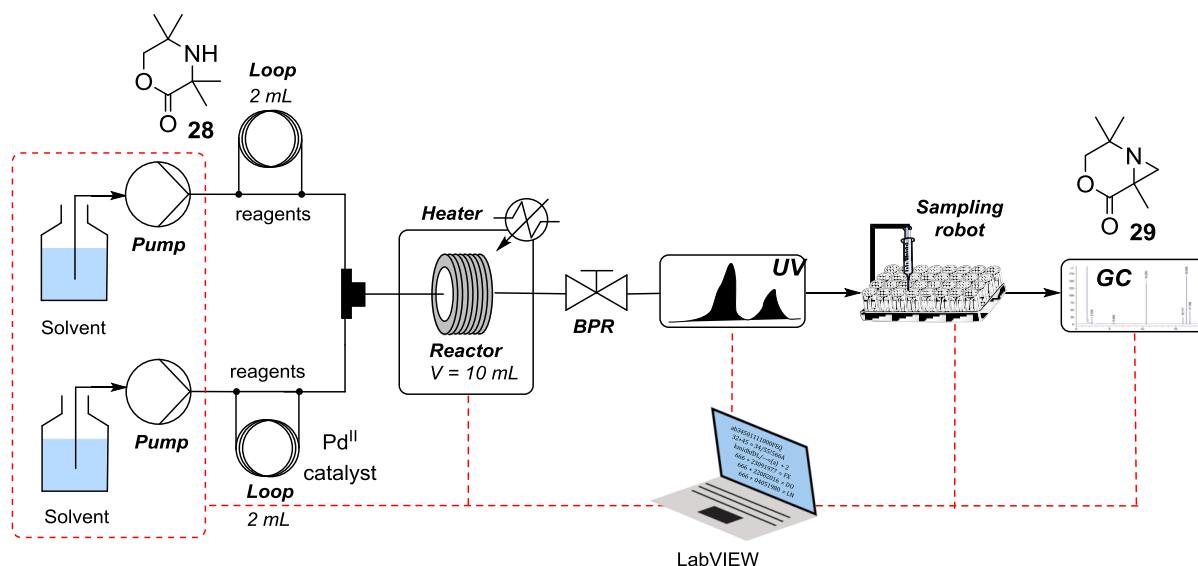
Low detection limits as well as fully quantitative data provided by GC analytical technique are important advantages in the identification of byproducts as well as in the discovery and optimization of unexpected reactions. Amara, Poliakoff and co-workers detected unusual nucleophilic substitutions while performing the methylation of aniline **22** with dimethyl carbonate in the presence of THF, a common organic co-solvent initially used to increase the

solubility of reagents in non-polar supercritical media (Scheme 17).<sup>94</sup> During this study the authors reported the mechanism and optimization of the synthesis of new unexpected *O*- and *N*-methylation derivatives **25-27**, with the latter being kinetically favored.



**Scheme 17.** Nucleophilic substitutions products from the catalytic reaction of aniline with DCM and THF in  $sc\text{CO}_2$ .<sup>94</sup>

A good example of analytical accuracy achieved using online GC analysis is the work presented by A. Echtermeyer, A. Lapkin and co-workers on the comparison of two optimization approaches, black-box self-optimisation vs model-based design of experiments strategy, for developing a C-H activation flow process.<sup>96</sup> The continuous flow system consisted of a commercial Vapourtec R2+/R4 system connected to an in-line UV cell for the detection of the sample segments and an online GC for the analysis of the composition of the outcoming segments (Scheme 18). GC analysis of aziridine **29**, obtained from the palladium-catalyzed C-H activation of amine **28** was performed with an impressive accuracy of  $\pm 0.0005$  mol L<sup>-1</sup>. The authors concluded that while model-based design of experiments approach allowed a rapid generation of a kinetic model, the self-optimising strategy driven by a black-box algorithm appeared to be extremely efficient and needed only a very few number of experiments to reach the optimum.



**Scheme 18.** Self-optimizing flow reactor with integrated UV detection and GLC analysis for palladium-catalyzed intramolecular C-H amination under continuous flow. Dashed red lines refers to computer-controlled units.<sup>96</sup>

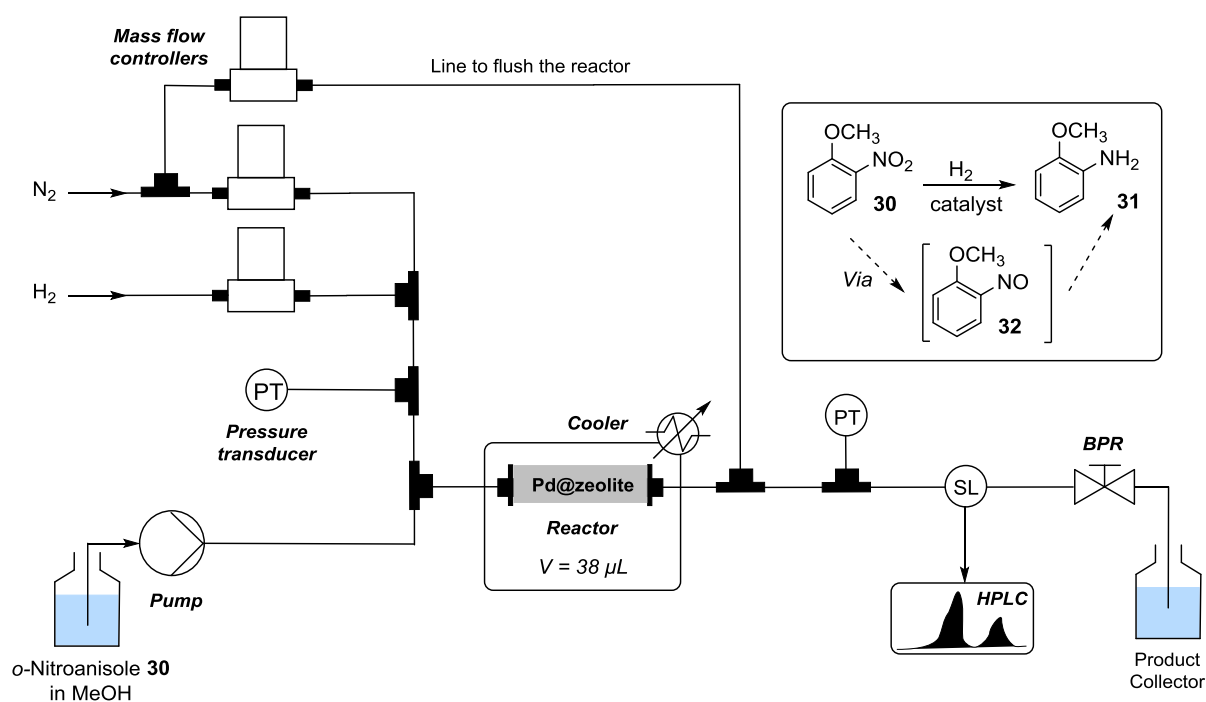
**Liquid Chromatography (LC).** Liquid Chromatography is often considered as the sister technique of gas chromatography. Although there are obviously several similarities with gas chromatography, liquid chromatography is more complementary than twin and has its own specificity. With liquid chromatography, the liquid sample is carried by a mixture of solvents (mobile phase) into a column containing a solid adsorbent (stationary phase). Different flow rates stem from the interaction of each analyte with the stationary adsorbing phase resulting in their separation through diffusion and mass transfer processes. The analyte-stationary phase interactions are markedly affected by the chemical nature of the analytes along with the temperature of the analysis, the composition of the mobile phase and the nature of the column. Quantitative analysis is assured by a selective or universal detector placed at the exit of the column measuring a physical or bulk property of the analytes (UV-Vis, mass spectrometry (MS), photodiode array (PDA) or refractive index among others).

**High-performance liquid chromatography (HPLC).** HPLC is a LC technique operating under high pressure (typically 50-400 bar). HPLC has become a very popular analytical technique due to its high resolving power when compared to ordinary liquid chromatography (LC) where the mobile phase is driven by the force of gravity to percolate through the stationary phase. Conventional HPLC presents many advantages: robustness, ease of use, well-known separation principles, good sensitivity and adjustable selectivity. The HPLC

column efficiency, also known as plate count (dispersion of peaks), increases with the use of particles with smaller diameter, even though, the use of such particles implies a rapid increase in pressure drop whereas most HPLC systems are limited to 400 bar. Therefore, short columns filled with particles of about 2  $\mu\text{m}$  can be used for conventional HPLC systems in order to speed up the analysis without losing efficiency and maintaining an acceptable pressure drop.

Depending on the application, isocratic or gradient elution methods can be selected for an efficient separation of the analyte peaks. Commonly employed detectors for HPLC analysis are Ultraviolet (UV) for light-absorbing compounds, evaporative light scattering (ELSD) or charged aerosol (CAD) for nonchromophoric compounds, and mass spectrometry (MS). The detectors identify the separated analytes and quantify their concentrations which are converted into electronic signals. UV detectors are the most widespread HPLC detectors used nowadays. UV detectors can operate at fixed wavelength, at multiple wavelength or driven by photodiode array sensors.

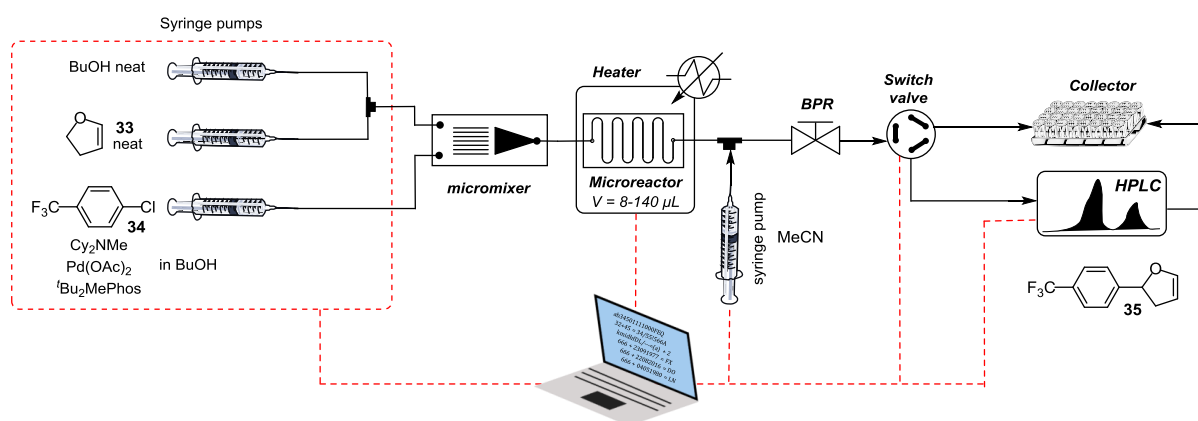
The integration of online HPLC detection in continuous flow systems represents a powerful tool for reaction development through the monitoring of experimental conditions and reaction progress. This strategy was implemented by Tadepalli and co-workers upon studying the kinetics of the Pd-catalysed hydrogenation of *o*-nitroanisole **30** to *o*-anisidine **31** in a flow fixed-bed microreactor connected to an online HPLC analyzer (Scheme 19).<sup>97</sup> This highly exothermic transformation was carried out under transitional Taylor flow regime. The efficient heat removal capacity of the microreactor prevented from temperature gradients and the emergence of side reactions. Online HPLC allowed to identify significant amounts of the 2-methoxynitrosobenzene **32** intermediate, directing the study towards the kinetic characterization of two reaction steps: the sequential reduction of *o*-nitroanisole **30** to 2-methoxynitrosobenzene **32** and the reduction of the latter to *o*-anisidine **31**.



**Scheme 19.** Flow platform for the catalytic hydrogenation of *o*-nitroanisole **30** to *o*-anisidine **31** integrating online HPLC analysis.<sup>97</sup>

Compared to in-line spectroscopic techniques (IR, Raman and NMR), HPLC analysis allows reproducible and easier analyte resolution from a multicomponent sample. Whereas spectroscopic techniques provide structural information, their spectra are plagued with overlapping peaks when analyzing reaction mixtures. Efficient HPLC separation enables quantitative analysis of each compound represented by a single chromatogram peak. This is particularly relevant when studying organic synthesis involving reagents and products with close chemical structures and/or regio- and stereochemically different compounds. Jensen, McMullen and co-workers have employed a silicon microreactor connected to a HPLC device to self-optimize several organic reactions of interest.<sup>98-101</sup> In a representative example, the palladium-catalyzed Heck reaction of 4-chlorotrifluoromethylbenzene **33** with 2,3-dihydrofuran **34** was optimized using an automated microreactor system with feedback control and optimization algorithms.<sup>99</sup> Three syringe pumps delivered the reagents in solution which met a 101-channel interdigitated micromixer to ensure the efficient mixing of the three streams (Scheme 20). The reaction occurred in a silicon microreactor connected to a six-port valve issuing 2 μL of the reaction mixture from the outlet of the reactor to the online HPLC analyzer. An isocratic HPLC method using methanol and formic acid in water was implemented. The reaction yield was determined by comparison with an internal standard and communicated to the algorithm which proposed new set of experimental conditions.

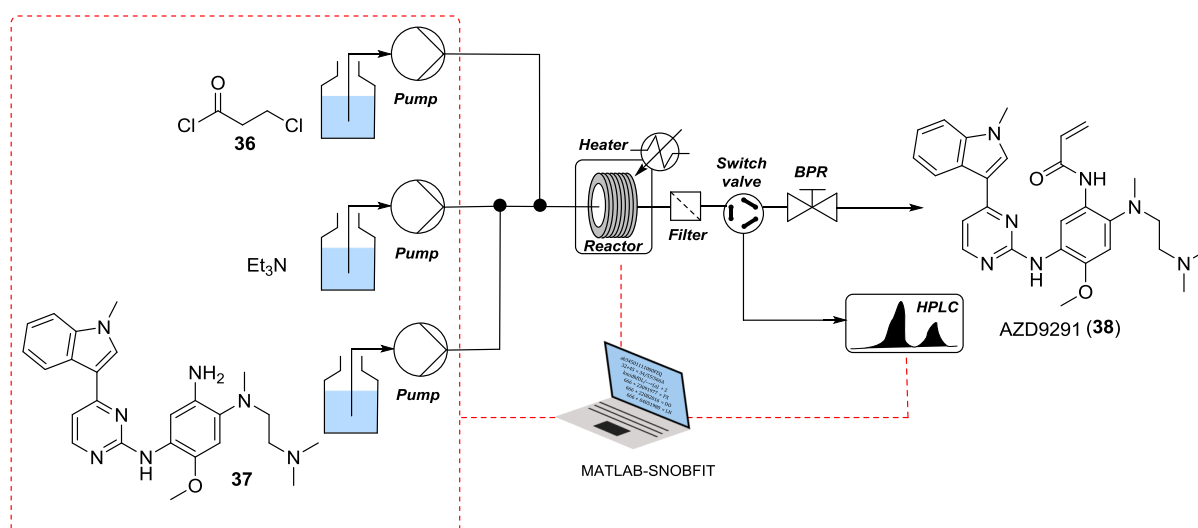




**Scheme 20.** Self-optimizing flow microreactor with integrated HPLC analysis for Heck type reactions. Dashed red lines refers to computer-controlled units.<sup>99</sup>

Generally, HPLC determination of the selectivity and the yield of a chemical process requires the formulation of a calibration curve relating the concentration of the species of interest to the area of the chromatogram measured.<sup>98-99, 102</sup> Numerical integration of the area of the absorbance signals from the generated chromatograms was ensured by either commercially available or ad-hoc control softwares (LabVIEW, MATLAB...) and computed to determine the concentration of the analytes of interest according to the calibration curve. In this frame, Bourne *et al.* used a variable wavelength detector and a gradient HPLC method for the calibration and optimization analysis involved in the self-optimisation/DoE of a Claisen-Schmidt condensation.<sup>66</sup> The authors faced critical calibration issues due to non-linear responses, requiring to develop a variable wavelength method. The variable wavelength detector selected the adapted wavelength for each analyte in order to quantify them within their respective linear detection limits, avoiding saturation issues.

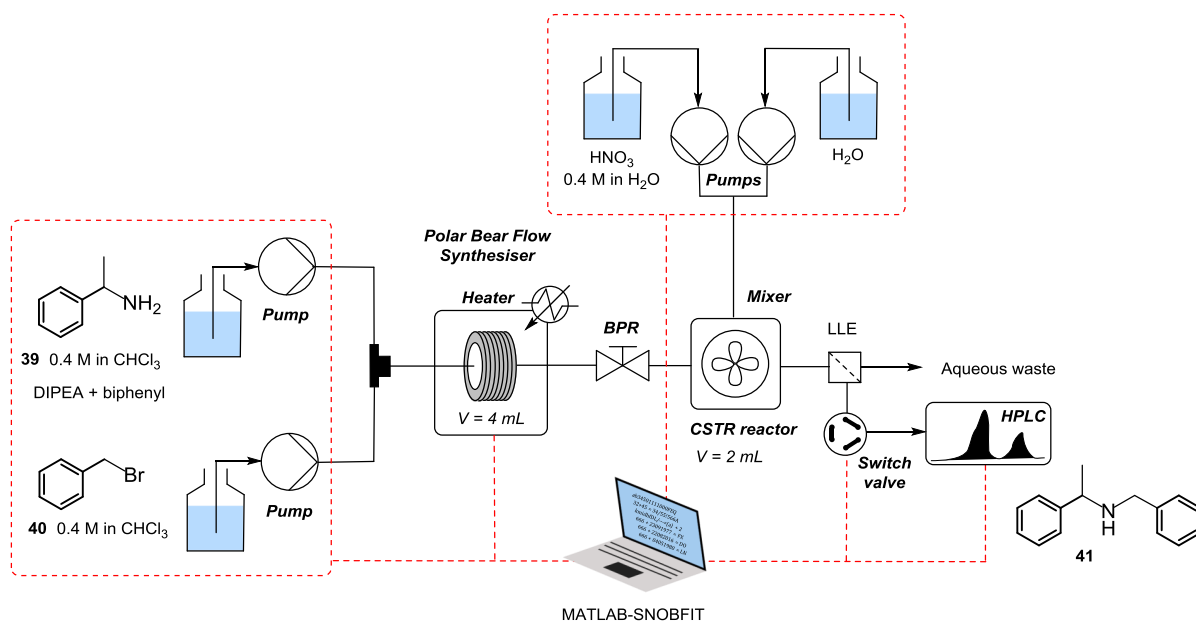
Compared to real-time analysis methods, online HPLC requires several minutes to get a chromatogram from a reaction mixture. However, this time may be not critical when optimization experiments can be set without requiring a real-time feedback. In this frame, Bourne *et al.* optimized the final stage in the synthesis of EGFR kinase inhibitor AZD9291 (**38**) using a self-optimizing flow reactor integrating a HPLC equipment (Scheme 21).<sup>103</sup> In their hands, the impact of the HPLC analysis on the overall optimization time was minor given that each new reaction started as soon as a sample of the previous experiment was delivered to the HPLC device.



**Scheme 21.** Flow self-optimizing reactor with integrated HPLC analysis final stage in the synthesis of AZD9291 (**38**) using the SNOBFIT optimization algorithm. Dashed red lines refers to computer-controlled units.<sup>103</sup>

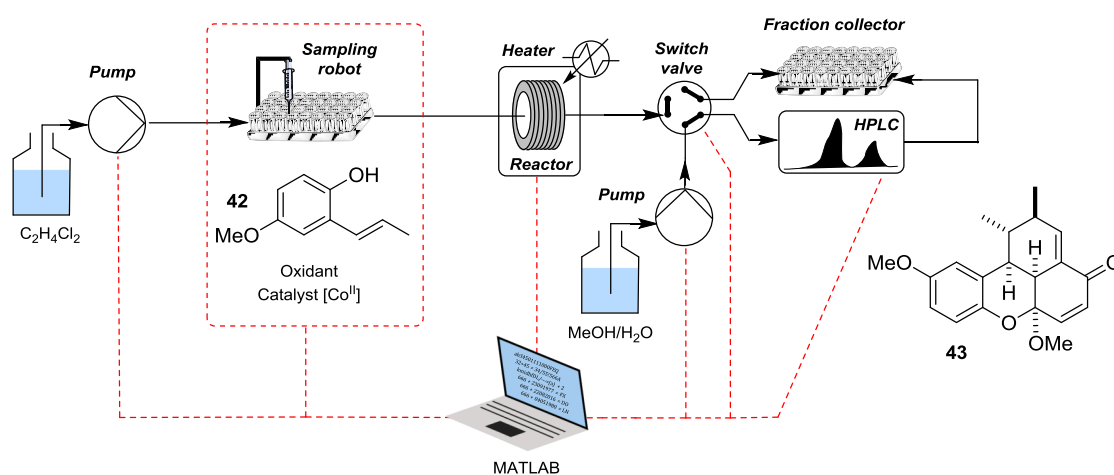
Particular attention when running online HPLC analysis concerns the compatibility of the stationary phase of the chromatographic column with the reaction medium when the latter is sampled directly at the reactor outlet without prior treatment and work-up. One solution for increasing the column lifetime consists in carrying out a downstream work-up to neutralize any reacting species that could damage the stationary phase.

The concept of in-line downstream work-up was advantageously exploited by Bourne et al. to selectively extract non-desired amines from the reaction mixture.<sup>104</sup> Impressively, they developed a reconfigurable continuous flow platform to simultaneously optimize the benzylation of  $\alpha$ -methylbenzylamine **39** with benzylbromide **40** and the pH-based liquid-liquid extraction (LLE) through a black-box optimization approach. The LLE was carried out in a continuous stirred-tank reactor (CSTR) for mixing liquid-liquid biphasic mixtures with independent flow rates (Scheme 22). The LLE step was optimized by adjusting the flow rates of three streams: the reaction output containing a mixture of amines, the stream of nitric acid used to protonate the non-desired amines, and a stream of water for dilution.



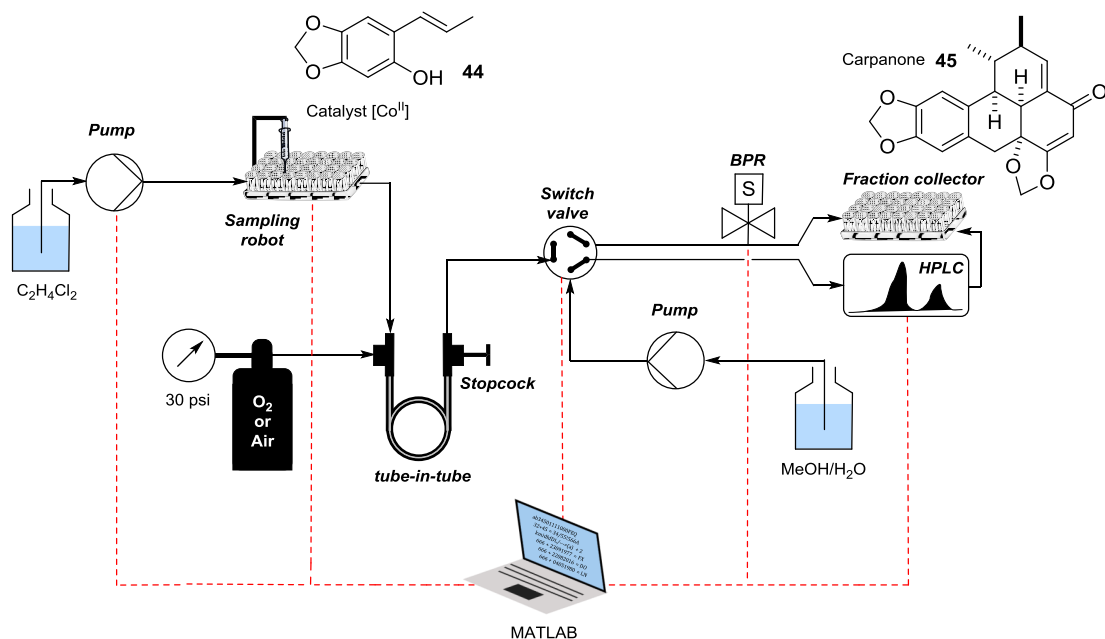
**Scheme 22.** Self-optimising reconfigurable continuous flow system for the tandem reaction-extraction process involving the synthesis of *N*-benzyl- $\alpha$ -methylbenzylamine **41** and its pH driven separation from structurally similar by-product amines. Dashed red lines refers to computer-controlled units.<sup>104</sup>

Further progress in chemical synthesis involving the optimization of both discrete and continuous variables has been achieved by integrating online HPLC analysis into an automated flow platform. In this frame, a reconfigurable flow platform was developed for the automated discovery of new chemical reactivities and the autonomous optimization of continuous variables in the cobalt-catalyzed synthesis of bioinspired lignans.<sup>105-106</sup> The flow device was first configured for the automated screening of a matrix of four catalysts and five oxidants at different temperatures on micromole scale affording one fully characterized reaction every 26 min (Scheme 23). The one-stream device included a sample handling robot for the preparation of the reaction mixture, a HPLC pump, a PEEK reactor coil, a sample loop and an online HPLC analyzer provided with a UV detector (270 nm). After the reagent screening campaign, the flow system was straightforward reconfigured to an autonomous self-optimising flow reactor driven by an optimization algorithm integrated in the process control experimental sequence. Continuous variables (residence time, temperature, catalyst loading, and equivalents of oxidant) were, in this way, optimized for the maximization of the yield and the productivity of the benchmark oxidative dimerization of 2-propenyl phenol **42** to benzoxanthone **43**. This study disclosed the potential of alternative oxidants such as *t*-BuOOH through a highly reproducible flow strategy.



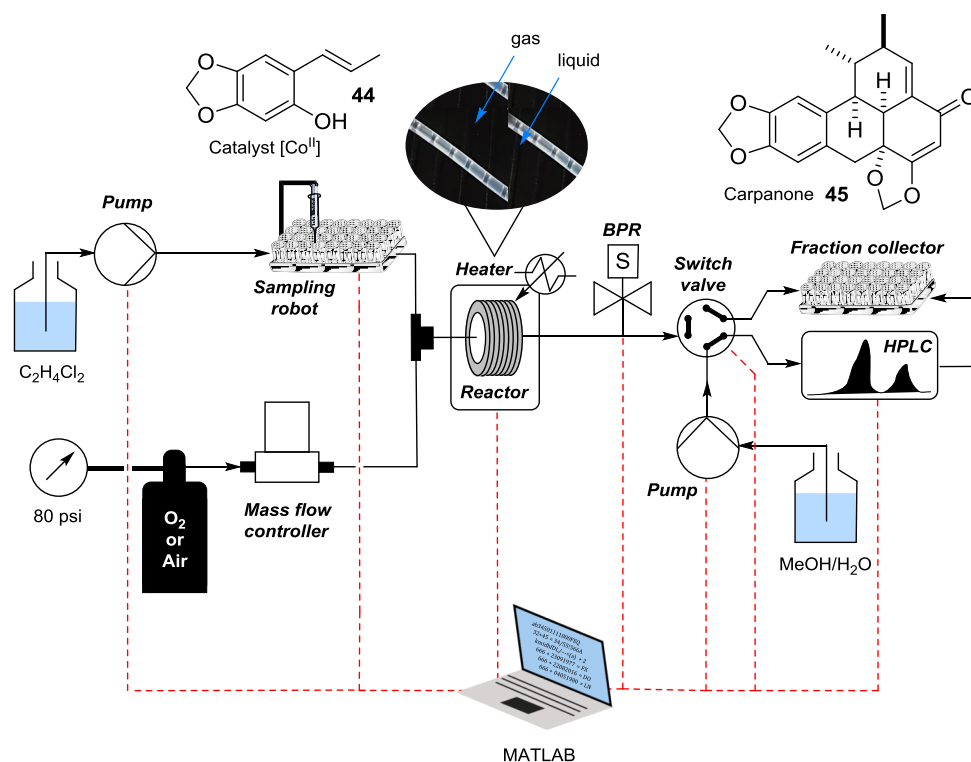
**Scheme 23.** Automatic screening platform for the evaluation of oxidizing conditions. Dashed red lines refers to computer-controlled units. Adapted from ref. 106 with permission from The American Chemical Society.

Nevertheless, molecular oxygen is an attractive sustainable oxidant and continuous flow reactors are particularly well suited to perform aerobic oxidations since most of the hazardous issues associated with the use of oxygen are mitigated. Two strategies are available in flow to conduct aerobic oxidations: gas-liquid segmented and tube-in-tube setups. These two approaches have been implemented for the study of the dimerization of desmethoxycarpacine **44** to carpanone **45** in the presence of oxygen with an automated continuous flow device integrating an online HPLC analyzer (Scheme 24).<sup>107</sup> The tube-in-tube platform included a sampling robot for preparing the reaction mixtures from stock solutions, a pump, a reactor consisting of Teflon AF-2400 tubing inserted into 1/8" ETFE tubing, an automated switch valve, a back pressure regulator and an online HPLC analyzer with a UV detector (254 nm).



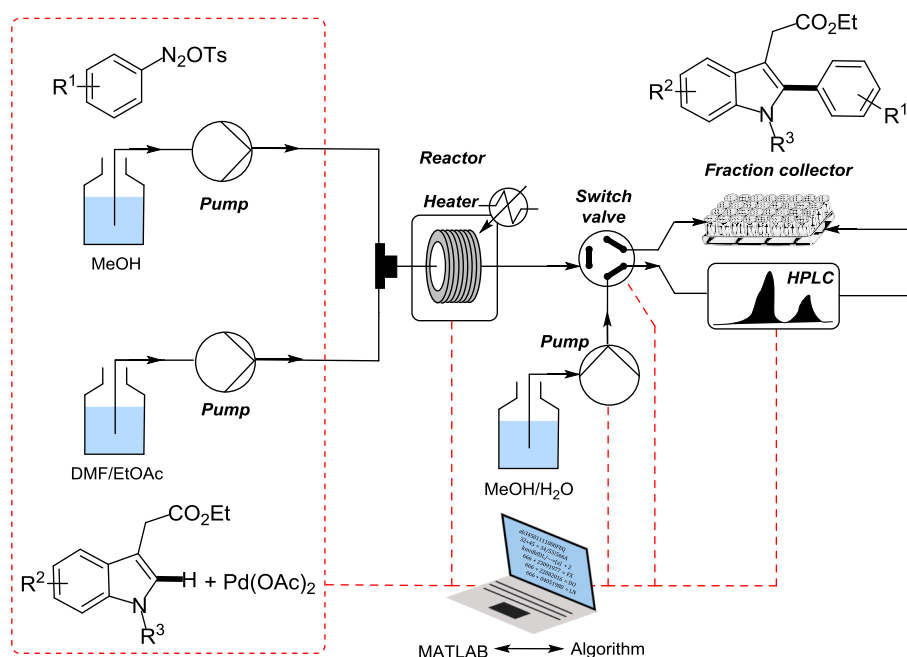
**Scheme 24.** Automated tube-in-tube platform. Dashed red lines refers to computer-controlled units. Adapted from ref. 107 with permission from The American Chemical Society.

In the reconfigured gas-liquid segmented flow platform, the tube-in-tube device was replaced by a single ETFE tube (Scheme 25).<sup>107</sup> In this case, the delivery of gas was precisely regulated by a mass flow controller. Evidenced by online HPLC analysis, deactivation of the catalysts rather than gas-to-liquid mass transfer was identified as the rate-limiting step affecting the yield of this transformation. Higher HPLC yields were measured with the gas-liquid segmented approach which revealed more robust than the tube-in-tube setup.



**Scheme 25.** Automated gas-liquid segmented platform. Dashed red lines refers to computer-controlled units. Adapted from ref. 107 with permission from The American Chemical Society.

Online HPLC coupled to a continuous flow device driven by a process control software and an optimization algorithm, has also offered the possibility to self-optimize transformations involving hazardous chemicals such as diazonium salts. With this aim, an operationally simple, robust, safe and efficient two-stream flow platform was developed by assembling two pumps, a stainless steel reactor coil placed in an oven, a switch valve and an online HPLC analyzer, the whole system being managed by an in-house process control software (MATLAB) including a custom made optimization algorithm (Scheme 26).<sup>80, 108</sup> The methodology afforded a broad substrate scope under mild conditions and without neither additives nor classical offline manipulation as products were analyzed online, yields calculated automatically and the new experimental conditions set by the algorithm.



**Scheme 26.** Autonomous self-optimizing flow platform for the study of a Heck-Matsuda type C-C coupling.<sup>108</sup>

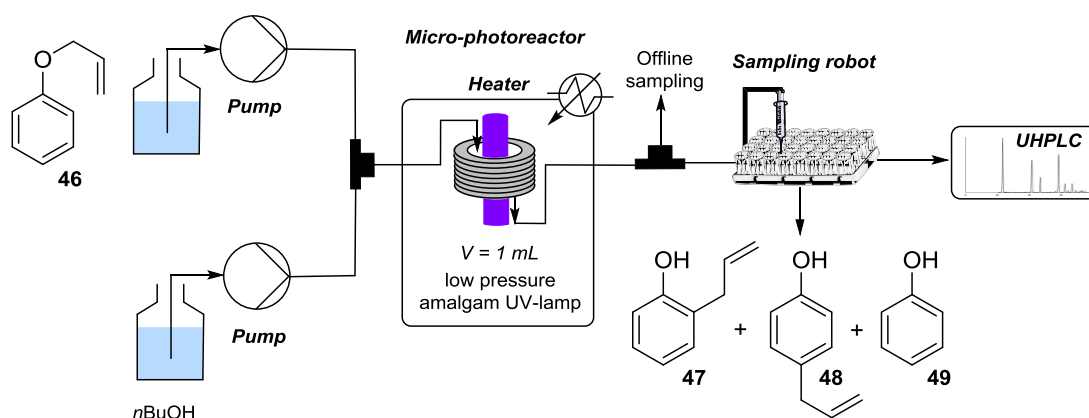
Besides, combining online analyzers with feedback control devices and logic algorithms, kinetic parameters of a process can be assessed by estimating the rate equation of a reaction and the parameters of the rate constants. This was successfully conducted at micro- and milliliter scale by a numbering up procedure using designed silicon reactors.<sup>109-110</sup> Kinetics parameters were determined as of analyte concentrations from 2  $\mu$ l diluted aliquots of the reaction product measured by online HPLC equipped with a PDA detector operating with a gradient method. While Jensen and co-workers<sup>110</sup> successfully used a steady-state method, Bourne *et al.*<sup>111</sup> developed a transient approach based on the flow ramp technique, saving time and chemicals with similar reliability.

**Ultra-High Performance Liquid Chromatography (UHPLC).** The efficiency and the analysis timescale of HPLC separations could be further improved by i/ working at higher temperature to lower the viscosity and boost the diffusion of the analytes, resulting in lower pressure drop and better efficiency, ii/ using monolithic columns as to avoid limitations due to pressure drop effects, iii/ implementing UHPLC systems.

UHPLC technique relies on the use of a stationary phase made of particles (<2  $\mu$ m) smaller than in HPLC columns (3-5  $\mu$ m). As a result, it is possible to use a much wider range of flow rates, to increase the throughput, and therefore to speed the analysis without altering the

chromatographic performance. UHPLC requires, however, equipment compatible with much higher generated pressures (550 to 1000 bars vs 100-400 bars in HPLC) and capable of taking advantage of the separation performance by limiting dead volumes. Moreover, the increase in pressure entails more maintenance and reduces the life of UHPLC columns which generally cannot be regenerated, notably with particles smaller than 2  $\mu\text{m}$ .

A pivotal advantage of online UHPLC equipment is the ability to analyze organic systems within few seconds to several minutes,<sup>112</sup> therefore reducing the effect of dispersion upon determining kinetic parameters with the flow ramp approach in continuous flow reactors. Continuous online fast sampling and shortened timeline of measurements using UHPLC were important features for the monitoring of the photo-Claisen rearrangement of allylphenylether **46** to phenols **47-49** in a continuous flow capillary micro-photoreactor.<sup>113</sup> The flow platform developed by Hessel *et al.* consisted of two HPLC pumps merging into a T-mixer fit to the inlet of FEP capillary photoreactor linked to a modified UHPLC system through a T-piece allowing for offline sampling as well (Scheme 27). The flow setup included three interconnected units for online sampling (40  $\mu\text{L}$ ), automated dilution (100  $\mu\text{L}$ ) and UHPLC analysis (aliquot of 1  $\mu\text{L}$ ). Analytical isocratic separation was performed using a C18 UHPLC column, and detection was carried out with a UV-Vis diode array detector. The novel analytical approach, known as Quality-In(Process)Line (QuIProLi), proved to be repeatable, reproducible and adapted to fast transformations, very low concentration mixtures and accurate measurement requiring low volumes of analytes.



**Scheme 27.** Micro-flow UV-photo Claisen rearrangement with integrated online UHPLC.<sup>113</sup>

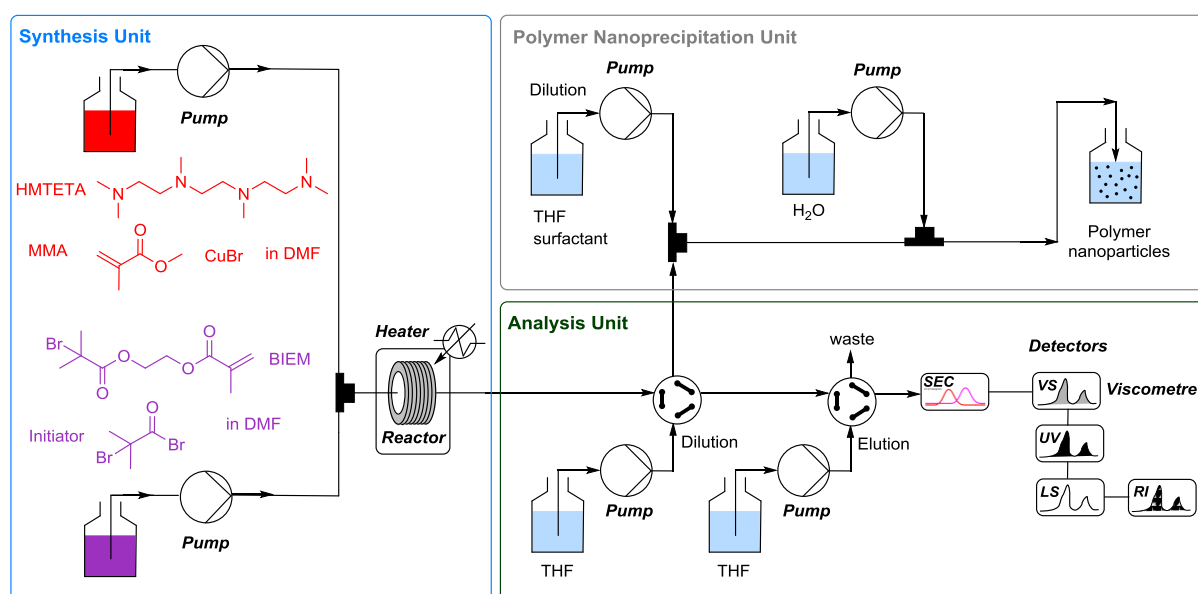
**Size-exclusion chromatography (SEC).** A related liquid chromatography technique is Size-Exclusion Chromatography (SEC), a low resolution chromatographic method which separates analytes according to their molecular size and/or molecular weight, commonly used



for the analysis of large molecules which do not enter the pores of the solid stationary phase (porous polymer beads). The larger the molecules, the faster the elution and the shorter the retention time, as too large particles cannot enter the pores of the stationary phase and stay in the void volume. Considering that no interaction between the solute and the stationary phase is required in SEC, the analytes can be recovered without loss, provided that a non-destructive detector is integrated. Once the mobile phase has passed through the column, the filtered solution (eluate) is analyzed by spectroscopic techniques (refractive index, ultraviolet) in order to determine the concentration and the size of the eluted analytes. Nevertheless, other techniques may be necessary to accurately characterize the components of each fraction. When coupled to a light scattering detector, the SEC technique benefits from a gain in sensitivity affording narrow bands and short and well defined separation times. Reproducibility of SEC is commonly set on the order of few percent and is typically considered as a filtration/permeation chromatography since large molecules can be well separated from small molecules with a minimal volume of eluate.

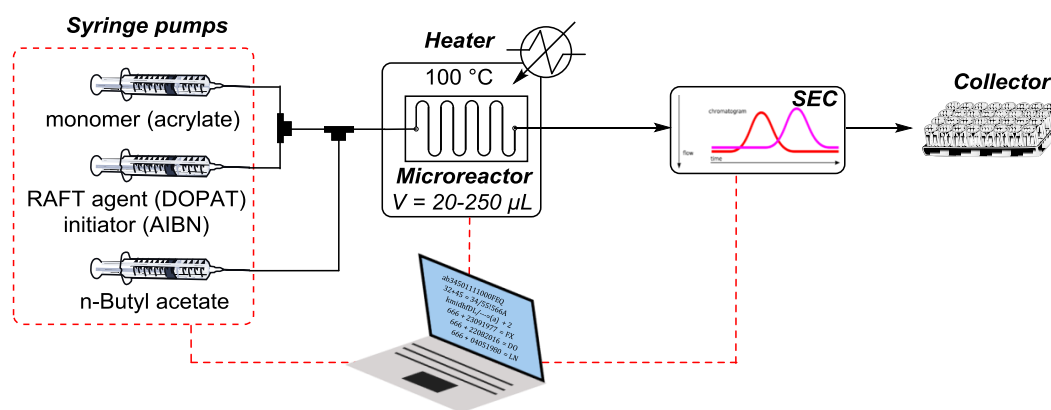
The integration of online analysis has played a pivotal role in the monitoring and optimization of polymerizations, known to be highly sensitive to impurities and changes in the reaction conditions, through fast kinetic screening. In this area, online SEC analysis has been advantageously employed.<sup>114-116</sup> For example, the production of polymer particles from monomers through a continuous flow process involving sequential synthesis, analysis and precipitation steps, was monitored with online SEC chromatography.<sup>114</sup> The microplant design depicted in Scheme 28 includes three main units: i/ the polymer synthesis block, ii/ the polymer characterization unit and iii/ the polymer precipitation setup. The synthesis unit consists of two HPLC piston pumps bringing the reagent to a thermoregulated stainless steel microtube reactor connected, through several sampling and injection valves, to the characterization unit composed of a gel permeation chromatography (GPC) apparatus integrating four online detectors (UV, single capillary viscometer, dual-angle light scattering, and refractive-index). Polymers with different molecular weight were separated by the GPC and analyzed by the train of detectors. Comparing the molecular weights given by the refractive-index and the light scattering detector, it was possible to determine qualitatively the branching rate of the polymer. The last unit of the continuous microfluidic platform was dedicated to the in-line micromixer-assisted polymer nanoprecipitation step which required a set of pumps and macro/micromixers placed downstream to the outlet of the reactor in order to dilute, mix and nanoprecipitate the synthesized polymer aiming at its isolation and purification. Online multi-detection gel permeation chromatography allowed the

characterization of linear and branched polymers and the preparation of 100 nm size particles by tuning the process parameters. Online analysis enabled to study the dynamics of the reactor in order to determine macromolecular parameters such as the steady state of the reactor for a specific type of polymerization (linear, branched, etc). Using this strategy, drug delivery applications were considered.



**Scheme 28.** Continuous microflow platform for the synthesis, characterization and recovery of polymer nanoparticles.<sup>114</sup>

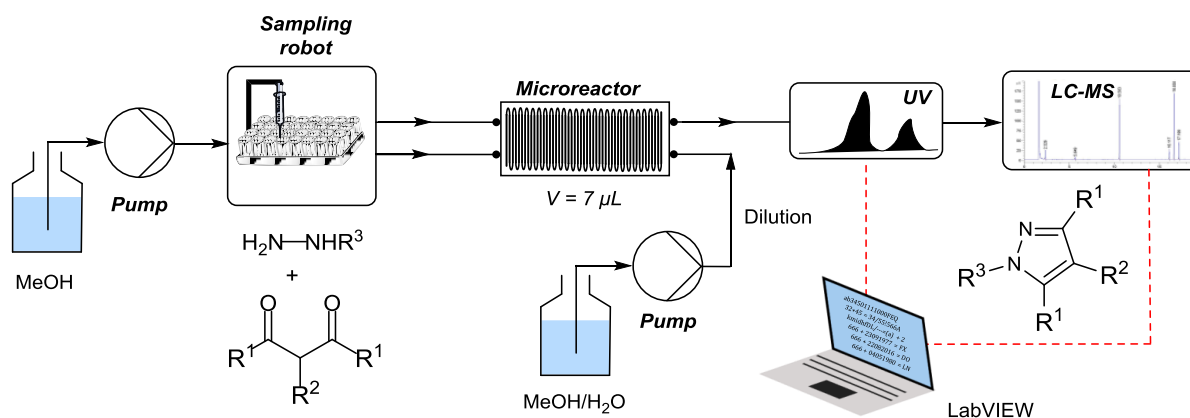
In another innovative work, Junkers and coworkers reported the first autonomous self-optimizing flow reactor integrating an online SEC for the monitoring of controlled polymer synthesis (Scheme 29).<sup>116</sup> Their autonomous platform enabled a remarkably reproducible and accurate (< 2.5 % deviation) optimization of the average molecular weight of targeted size polymers using machine-learning algorithms. The coupled SEC analyzer was equipped with an evaporative light scattering detector which provided directly relevant molecular weight information (concentration and particle size/molecular weight).



**Scheme 29.** Self-optimizing platform for the thermal RAFT polymerization of acrylates. Dashed red lines refers to computer-controlled units.<sup>116</sup>

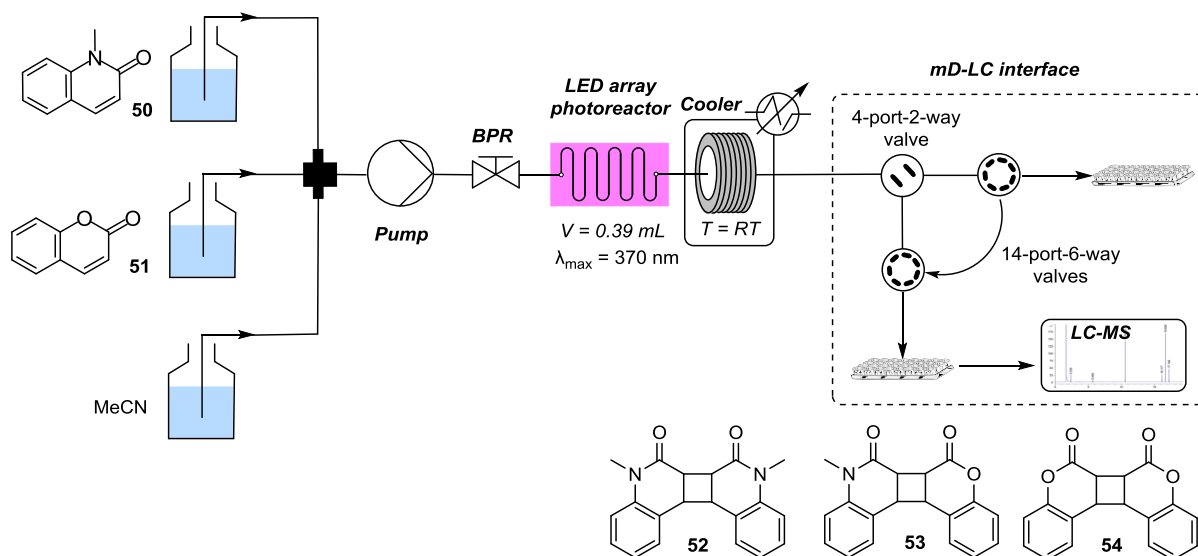
### Multiple in-line/online analytical tools.

The non-universality of a single analytical tool is an acute issue for the monitoring of complex reaction mixtures. The integration of multiple analytical sources into flow reactors is a powerful solution to monitor flow processes in data-rich environments.<sup>117</sup> For example, the use of liquid chromatography coupled with mass spectrometry combines the separation and quantification ability of the former with the identification data provided by the latter. In this frame, LC-MS devices have been integrated in automated flow reactors for the online monitoring of chemical reactions.<sup>118-121</sup> For example, Garcia-Egido and co-workers employed an automated single-channel glass microreactor system equipped with an online LC-MS analyzer to study the Knorr reaction of 1,3-dicarbonyl compounds with hydrazines to prepare a library of pyrazoles (Scheme 30).<sup>118</sup> A UV detector was used to detect the slug at the chip outlet and trigger the LC-MS analyzer. This system allowed the automatic synthesis of combinatorial libraries (21 reactions) in a sequential way without neither cross-contamination between consecutive reactions nor offline manipulation for characterization.



**Scheme 30.** Automated single-channel glass microreactor “radiator” system equipped with an online LC-MS analyzer. Dashed red lines refers to computer-controlled units.<sup>118</sup>

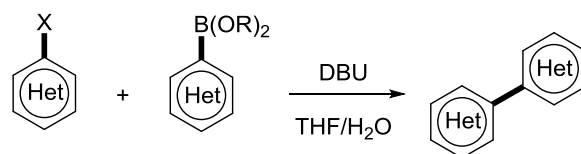
The powerful combination of liquid chromatography with mass spectrometry is penalized by the time required for chromatographic separations which does not correspond to the time scale of most chemical reactions; mass spectrometry being on its own considered as real time. This issue was recently addressed through the development of the so-called multi-dimensional liquid chromatography (mD-LC).<sup>122</sup> The novelty lies on the use of online 2D-LC-MS analyzers where the two timescales related to the analysis (sampling and acquisition data) were decoupled with a special valve assembly (mD-LC) enabling the parking of up to 10 samples (first dimension) exiting the photoreactor, independently from the progress of the chromatographic separation (second dimension). The introduction of multi-dimensional liquid chromatography was mainly motivated to ensure i/ higher resolution power to separate challenging samples and ii/ to provide an online desalting option for an easier coupling of MS detectors, which do not tolerate high concentrations of salts. The mD-LC interface has been integrated in a continuous flow microreactor equipped with an online-coupled HPLC/MS system.<sup>123</sup> Photochemical homo- and cross-dimerization reactions of quinolinone **50** and coumarin **51** to dimers **52-54** were investigated in systematic reaction parameter screenings (Scheme 31). Performing transient flow experiments requiring high sampling rates and high resolution HPLC analysis demanding longer acquisition times, were both possible in this way.



**Scheme 31.** 2-Dimension continuous-flow platform integrating a photo-microreactor and an online HPLC-MS analyzer equipped with a mD-LC interface for the study of [2+2] cycloaddition reactions.<sup>123</sup>

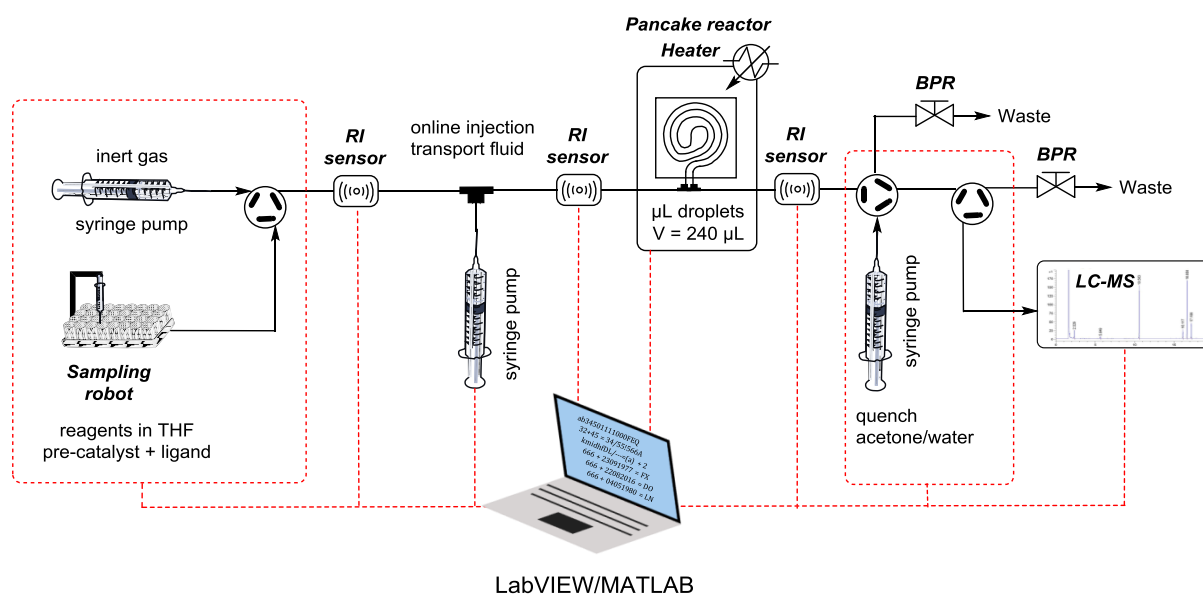
While LC-MS systems are essentially used to separate (LC) and identify (MS) target compounds from reaction mixtures, the possibility to tune the LC system as a preparative device to purify and isolate is a powerful asset to prepare libraries of pure compounds. In this frame, Tarver *et al.* designed an integrated discovery flow platform for the synthesis, purification, quantification and bioassay of a series of xanthine-derived dipeptidyl peptidase 4 antagonists.<sup>124</sup> They used a commercial LC-MS spectrometer equipped with an evaporative light-scattering detector (ELSD) to determine sample concentration and reformat the purified samples to the correct concentration for biological assay.

The high sensitivity of both liquid chromatography and mass spectrometry allows to analyze microliter-scale droplets flowing in high-throughput microfluidic systems. The Jensen group has strongly contributed to this field of research for reaction development.<sup>25, 100, 119, 125-127</sup> For instance, an autonomous droplet-flow microfluidic system integrating an online LC-MS analyzer was developed to explore and optimize palladium-catalyzed Suzuki-Miyaura reactions (Scheme 32).<sup>100</sup> The flow device was equipped with refractive index sensors to ensure correct timing of the sample injection in the online analyzer and the adequacy of the droplet volume. The synergistic effects of discrete (precatalysts and ligands) and continuous variables (temperature, reaction time, catalyst loading) were simultaneously considered through the optimization process to increase the chemists' understanding of the reaction. The capacity of LC/MS devices to analyze microliter-scale droplets allowed to run up to 96 experiments while limiting the amount of reagents and solvent required.



Discrete variables : [Pd], ligand

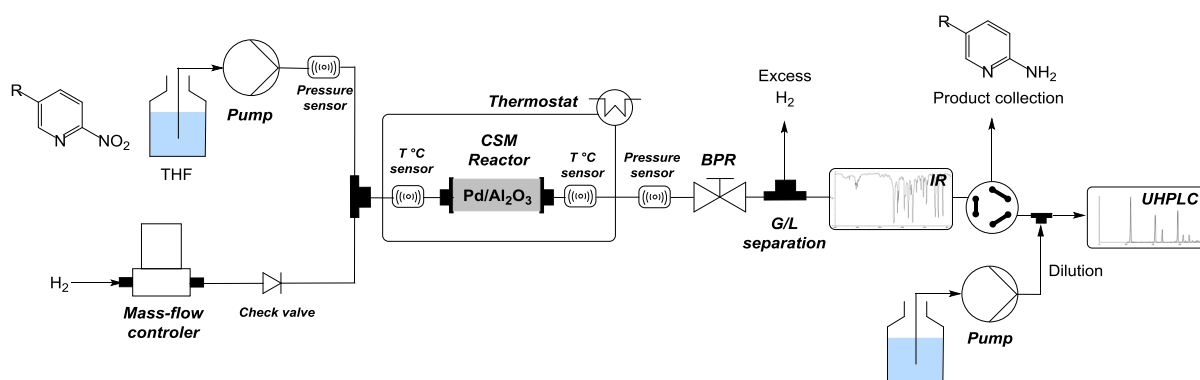
Continuous variables : Pd loading, temp., residence time



**Scheme 32.** Autonomous microliter-scale droplets flow system integrating LC-MS analyzers for the optimization of Suzuki reactions. Dashed red lines refers to computer-controlled units.<sup>100</sup>

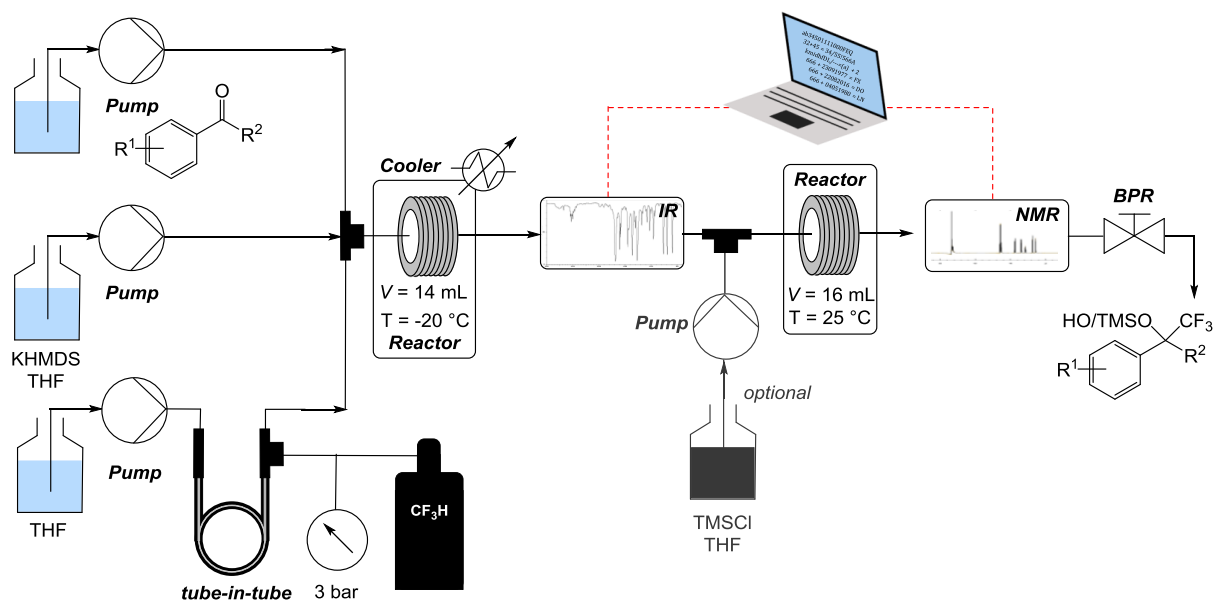
While the commercial availability of LC-MS systems from several manufacturers greatly facilitate their integration in flow platforms, other combinations of analytical tools have been occasionally reported.

In the search of an industrially viable solution for catalytic aromatic nitro reduction of a drug intermediate, Kappe et al. deployed the catalytic static mixer technology and implemented a combined monitoring approach consisting of in-line FTIR spectroscopy and online UHPLC (Scheme 33).<sup>128</sup> While FTIR could differentiate reagents and products and quantify concentrations and amounts of produced water, UHPLC gave a reaction progress overview. Thanks to 72 h FTIR data and >1100 online UHPLC samples, catalyst deactivation issue was understood and reaction parameters were rapidly optimized. The optimal conditions were ultimately scaled up to a reactor with 16 catalytic static mixers, leading to an impressive space time yield of  $6.5 \text{ kg L}^{-1} \text{ h}^{-1}$ .



**Scheme 33.** Continuous flow platform FTIR and UHPLC instruments.<sup>128</sup>

Real-time monitoring of chemical reaction is particularly useful to mitigate the handling of hazardous reagents. In this frame, Ley et al. developed nucleophilic trifluoromethylation of carbonyl compounds in flow using trifluoromethane ( $\text{CHF}_3$ ), a greenhouse gas with a lifetime of 270 years and generated as byproduct in the industrial manufacture of polytetrafluoroethylene (PTFE).<sup>129</sup> Converting this toxic waste material into high-value synthetic compounds would significantly improve the sustainability of the PTFE production. The homogeneous gas-liquid reactions were carried out at  $-20\text{ }^\circ\text{C}$  in a PTFE coil flow reactor equipped with a cooling device (Scheme 34).  $\text{CHF}_3$  gas reagent was delivered using a tube-in-tube device in which the inner capillary was a gas-permeable membrane (Teflon AF-2400). The reaction mixture was sequentially monitored downstream of the flow reactor by in-line FTIR and in-line benchtop  $^{19}\text{F}$  NMR (43 MHz). The combined analytical tools connected at the flow reactor outlet permitted the accurate dosing of  $\text{CHF}_3$  gas and the quantitative trapping of the corresponding anion. The in-line FTIR spectrometer was used to monitor the bands at  $1128\text{ cm}^{-1}$  ( $\text{CHF}_3$ ) and  $1328\text{ cm}^{-1}$  ( $\text{PhCF}_3$ , internal standard) until the steady state was reached. At this point, the NMR spectrometer takes over to provide information on the reaction progress through the recording of  $^{19}\text{F}$  spectra.

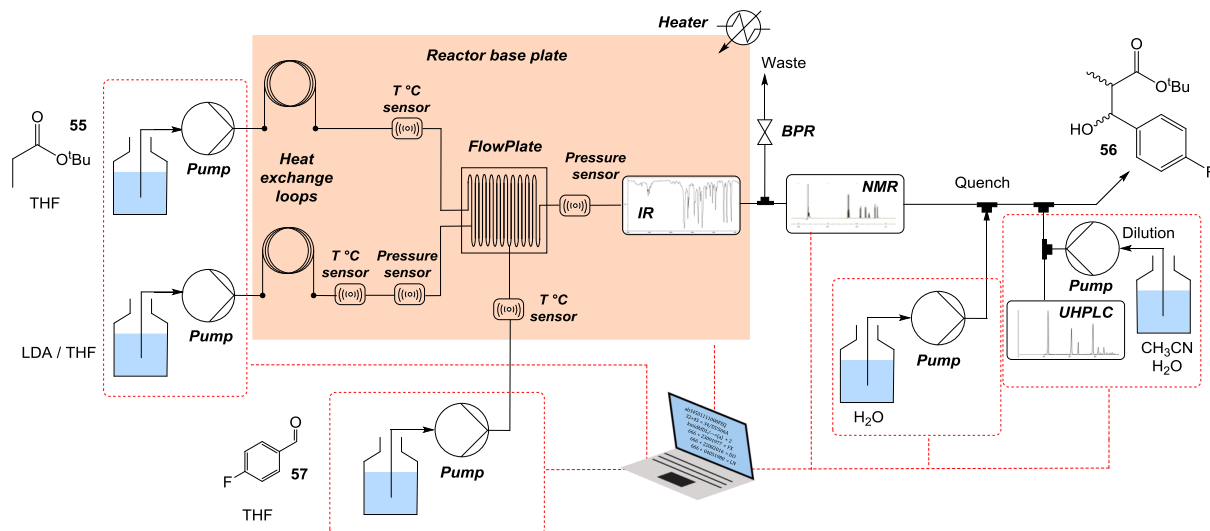


**Scheme 34.** Continuous flow platform integrating a PTFE coil flow reactor, a tube-in-tube  $\text{CF}_3\text{H}$  feeding module and real-time IR and NMR in-line PAT tools. Optional grey modules were used for in-line derivatization of alcohols. Dashed red lines refers to computer-controlled units.<sup>129</sup>

One very recent and emerging research area concerns the development of flow platforms allowing to perform multistep transformations without manual intervention.<sup>130</sup> Such flow platforms require monitoring of the transformation at multiple point within the flow stream by suitable analytical instruments. The group of Kappe has significantly contributed to this field of research.<sup>131-133</sup> As an example, they integrated three different in-line/online analytical instruments within a modular microreactor system for the optimization and in-process monitoring of a multistep organolithium transformation. The two-step transformation of *tert*-butyl propionate **55** into aryl propionate **56** was performed in a continuous flow Modular Micro Reaction System (MMRS).<sup>131</sup> The device included a 0,44 mL flow reactor, temperature and pressure sensors, a pumping system and three PAT techniques: IR, NMR and UHPLC (Scheme 35). The deprotonation of *tert*-butyl propionate **55** by lithium diisopropylamide was followed and quantified by the disappearance of the C=O stretch at 1730  $\text{cm}^{-1}$  and the concomitant formation of the enolate at 1644  $\text{cm}^{-1}$ . The addition of the lithium enolate to 4-fluorobenzaldehyde **57** was monitored by in-line benchtop  $^1\text{H}$  NMR spectroscopy using the aldehyde proton at 9.7 ppm as distinct marker. UHPLC was implemented for monitoring and quantifying the *in situ* quench step with water, affording an accurate analysis of the final product composition. A scale-out experiment using the integrated analytical instruments



afforded the desired product in 70% yield and with a throughput of 4.2 g h<sup>-1</sup>. Overall, this work appoints the advantages of implementing different analytical techniques at multiple points of a global process to efficiently control and optimize a multistep transformation.



**Scheme 35.** Modular Micro Reactor System (MMRS) integrating several PAT. Dashed red lines refers to computer-controlled units.<sup>131</sup>

### *Analytical tool guide*

For comparison purposes the following analytical tool guide is proposed (Figure 4). This guide cannot be considered as universal as it is based on the own vision and background of the authors, but it should be useful in the decision-making process of chemists wishing to integrate an analytical instrument within a continuous flow reactor.

FTIR	Raman	UV-Vis	MS
<ul style="list-style-type: none"> <li>▪ Good sensitivity</li> <li>▪ Excellent time efficiency</li> <li>▪ Automation possible</li> <li>▪ No sampling required</li> </ul>	<ul style="list-style-type: none"> <li>▪ Excellent time efficiency</li> <li>▪ Automation possible</li> <li>▪ No sampling required</li> <li>▪ Useful for aqueous solutions</li> <li>▪ Non-destructive analysis</li> </ul>	<ul style="list-style-type: none"> <li>▪ Good sensitivity</li> <li>▪ Excellent time efficiency</li> <li>▪ Automation possible</li> <li>▪ Non-destructive analysis</li> </ul>	<ul style="list-style-type: none"> <li>▪ High sensitivity</li> <li>▪ Good time efficiency</li> <li>▪ High resolution possible</li> <li>▪ Determination of molecular weight</li> <li>▪ Can be associated to HPLC or GC</li> </ul>
<ul style="list-style-type: none"> <li>▪ Modest structure information</li> <li>▪ Quantification (requires calibration)</li> </ul>	<ul style="list-style-type: none"> <li>▪ Modest sensitivity</li> <li>▪ Can induce sample fluorescence</li> <li>▪ Partial structure information</li> <li>▪ Quantification (requires calibration)</li> </ul>	<ul style="list-style-type: none"> <li>▪ Sampling required to prevent saturation</li> <li>▪ Quantification (requires calibration)</li> </ul>	<ul style="list-style-type: none"> <li>▪ Sampling required to prevent saturation</li> <li>▪ Automation possible but difficult</li> </ul>
<ul style="list-style-type: none"> <li>▪ Poor resolution with mixture of compounds</li> <li>▪ Not adapted to aqueous solution</li> </ul>	<ul style="list-style-type: none"> <li>▪ Poor resolution with mixture of compounds</li> </ul>	<ul style="list-style-type: none"> <li>▪ No structure information</li> </ul>	<ul style="list-style-type: none"> <li>▪ Quantification very difficult</li> </ul>
Benchtop NMR	HPLC/UHPLC	GC	SEC
<ul style="list-style-type: none"> <li>▪ Good structure information</li> <li>▪ No sampling required</li> <li>▪ Quantification</li> <li>▪ Non-destructive analysis</li> </ul>	<ul style="list-style-type: none"> <li>▪ Good sensitivity</li> <li>▪ Automation possible</li> <li>▪ Good resolution</li> </ul>	<ul style="list-style-type: none"> <li>▪ Good sensitivity</li> <li>▪ Automation possible</li> <li>▪ Good resolution</li> </ul>	<ul style="list-style-type: none"> <li>▪ Automation possible</li> <li>▪ Information on molecular weight</li> <li>▪ Non-destructive analysis</li> </ul>
<ul style="list-style-type: none"> <li>▪ Time efficiency depends of pulse sequences</li> <li>▪ Automation possible but difficult</li> <li>▪ Analysis difficult at high flow rates (&gt;2 mL/min)</li> </ul>	<ul style="list-style-type: none"> <li>▪ Sampling required</li> <li>▪ Require knowledge for the column selection</li> <li>▪ Quantification (requires calibration)</li> </ul>	<ul style="list-style-type: none"> <li>▪ Sampling required</li> <li>▪ Require knowledge for the column selection</li> <li>▪ Quantification (requires calibration)</li> </ul>	<ul style="list-style-type: none"> <li>▪ Modest sensitivity</li> <li>▪ Mostly useful for polymers</li> </ul>
<ul style="list-style-type: none"> <li>▪ Poor resolution with mixture of compounds</li> <li>▪ Poor sensitivity</li> </ul>	<ul style="list-style-type: none"> <li>▪ No structure information (improved with HPLC-MS)</li> <li>▪ Modest time efficiency (improved with UHPLC)</li> </ul>	<ul style="list-style-type: none"> <li>▪ No structure information (improved with GC-MS)</li> <li>▪ Modest time efficiency</li> <li>▪ Limited to volatile compounds</li> </ul>	<ul style="list-style-type: none"> <li>▪ Low resolution</li> </ul>

**Figure 4.** Analytical tool guide

## ■ CONCLUSION

The integration of process analytical tools into continuous flow platforms allows the monitoring of chemical reactions with minimal or no manual intervention in a minimal amount of time. Data collected from analytical instruments are useful for a deep understanding of the studied chemical transformation and can be used to modify in

(quasi)real-time different reaction parameters. Integrated PAT tools also mitigate the risk associated with chemical transformations through an accurate control and quantification of intermediates involved in the reacting mixture. This asset is particularly important when hazardous reagents or unstable intermediates are involved in chemical transformations. Unfortunately, none of the commercially available analytical tools can be universally used for any kind of chemical reactions. In this review we discussed in depth the main assets and drawbacks of most available analytical tools that can be integrated within continuous flow platforms. The discussion is illustrated by numerous examples issued from recent literature.

Among the most frequently integrated PAT, FT-IR, Raman and benchtop NMR are non-invasive real-time spectroscopic techniques well suited to monitor the progress of a chemical composition providing high speed data acquisition. The potential qualitative and quantitative information (structure, yield, rates...) given by these techniques could be compromised by signal overlapping issues when studying complex reaction mixtures. Higher resolution and sensitivity and easier data representation than NMR and vibrational spectroscopies respectively, can be provided by in-line MS. Unfortunately, quantification is not reliable when analyzing multicomponent mixtures. UV-vis and fluorescence spectroscopic techniques are also considered as real-time PAT displaying much higher sensitivities with very low detection limits and capable of providing accurate quantitative data. However, they lack structural data and need compounds active in the UV or with fluorescence properties. The counterpart non-real-time chromatographic techniques (GC, HPLC) are unable to provide structural data and the analysis takes several minutes instead of few seconds. Among their most advantageous specificities, low detection limits, high analytical accuracy and ease analyte resolution from a multicomponent sample could be mentioned. Additionally, chromatographic separation enables accurate quantitative analysis and plays a pivotal role in the identification of analytes with close chemical structure such as regio/stereoisomer compounds. The timeline of chromatographic separations has been shortened (down to few seconds) and gained in efficiency with UHPLC analysis. Still, a sampling step is needed in order to divert a small fraction of the main flow stream that needs to be diluted before flowing into the chromatography column (online analysis).

The complementarity of spectroscopic and chromatographic analytical techniques has naturally appeared as a very interesting strength for monitoring chemical processes. The association of chromatography techniques (LC, HPLC, UHPLC) with spectroscopic tools (MS, FT-IR, NMR) combines efficient separation, precise and reliable quantification and

structural identification abilities, representing a useful strategy for optimizing and monitoring multistep reactions and transformations in data-rich environments.

The integration of PAT tools combined with the use of algorithm and process-control software also pave the way for automated, even autonomous, chemistry 4.0.

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### Notes

The authors declare no competing financial interest.

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