6
Optical In-line Spectroscopy in Microchemical Processes

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6.1
Introduction

In general, the application of microfluidic devices in chemical and pharmaceutical synthesis can be roughly divided into two areas: *microchemical processing* and *lab-on-a-chip* applications.

Microchemical processing mainly emphasizes the exploitation of the well-known benefits of miniaturization such as very good heat and mass transport characteristics in a continuous process [1]. Microfluidic devices are used for process understanding, process optimization and development and finally production of specific products. The main reasons for employing microchemical processes on the laboratory and industrial scales are the following:

- process intensification (e.g. in terms of increased conversion and space–time yield, reduced by-product formation and improved product quality, reduction of waste and energy consumption);
- improved safety, for example due to smaller hold-ups and improved heat control.

On the other hand, microfluidic devices for lab-on-a-chip applications are mainly used in the context of analysis and diagnostics, often integrated in so-called *miniaturized total analysis systems* (μ-TAS) [2]. The fundamental idea of μ-TAS is to integrate all analytical steps such as sampling, sample pretreatment, analyte separation and detection for qualification or quantification within one device. Depending on the complexity of the sample, a lab-on-a-chip device can be a "simple" sensor, a flow-injection analysis (μ-FIA) or a complete analytical separation device such as a chromatographic (μ-HPLC) or a capillary electrophoresis (μ-CE) system [3].

Some researchers go one step further and propose a combination of a total analysis system with a chemical reaction during transportation: Two reagents or even two sets of different reagent classes react after mixing and are subsequently separated by one of the two above-mentioned miniaturized separation techniques [4]. In that way, high-throughput experimentation of *many different reactions* for setting up building block libraries for the pharmaceutical industry is accessible.

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In contrast to the wide variety of lab-on-a-chip technologies, the integration of analytical tools into *microchemical processes* has gained only minor importance until now, although appropriate in-line analysis has the potential to provide valuable information on the microchemical process performance. For example, a series of in-line spectra provide an insight into the fluid dynamics within the microfluidic devices or the uniformity of steady-state conditions. Knowledge about the chemical reaction pathways can be derived by the spectroscopic identification of reactive species and individual components can be monitored during the course of reaction. Although all non-concentration-based information is mainly of interest for process optimization purposes, the quantification of compounds in real time is highly relevant for industrial microchemical production. Recently, in-line spectroscopy for real-time quality control became an important issue in the pharmaceutical industry as a result of the PAT initiative of the US Food and Drug Administration (FDA). The goal of PAT (process analysis techniques) is to enhance the *understanding* of the manufacturing process and to ensure a constant quality of the product [5].

Currently more challenging is the integration of in-line analytics into a statistical process control system (SPC) [6]. As part of an automated microreaction process, this would allow active regulation of the process and would result in long-term quality, robustness and safety.

This huge potential makes the neglect of process analysis in microprocess engineering even more astonishing. Today, only very little literature is available that describes the use of PAT in microchemical processes. It seems that their obvious advantages have not yet been noticed, although the integration of PAT units into microreactors is well known in the μ-TAS community.

Therefore, the aim of this chapter is to present some of the capabilities of in-line analytical techniques on basis of optical spectroscopy adapted to liquid microreaction processes.

6.2
Optical Spectroscopy in Microchemical Processes

6.2.1
Spectroscopic Methods

Depending on the type of reaction and analytes to be investigated, different optical spectroscopic methods are available [7]. Typically UV–Vis, near-infrared (NIR), mid-infrared (MIR) and Raman spectroscopy are the most popular in process analysis (see Table 6.1) [8]. Other, emerging in-line methods such as fluorescence and chemiluminescence spectroscopy are still of minor importance in process technologies and will be not discussed here (for additional information, see [9]).

UV–Vis spectroscopy is an analytical method that measures molecular electronic transitions to characterize mainly organic molecules. For applying this method, the investigated species (analyte) has to absorb in the UV or visible wavelength range (200–780 nm). Consequently, this method is restricted to certain compounds such as

Table 6.1 Comparison of the different optical spectroscopic techniques.

	UV–Vis	NIR	MIR	Raman
Wavelength (nm)	200–780	780–2500	2500–50 000	2500–200 000
Type of interaction	Absorption (electronic transition)	Absorption (overtones, combination vibration)	Absorption, (fundamental vibration)	Inelastic laser light scattering, (fundamental vibration)
Physical requirement	Electronic transition must be within wavelength range	Overtone vibrations dominated by C−H, O−H, N−H	Change of dipole moment during vibration	Change of polarization during vibration
Information content	o	o	+	+
sensitivity	+	o	+	−
Integration via fiber optics	+	+	o	+
Usability in aqueous phase	+	o	o	+
Investment costs	+	o	o	−

− bad
o adequate
+ good

highly conjugated molecules or to charge-transfer complexes. UV–Vis spectroscopy is a very sensitive method but absorption bands are broad. Therefore, when analyzing mixtures, superpositions of different absorption bands limit this method to certain applications. In these cases, chemometric techniques may help to distinguish the different constituents.

In contrast to UV–Vis spectra, vibrational spectra detected in the MIR range and by Raman spectroscopy are highly specific and very sensitive to molecular structures. In both cases, vibrational bands can be assigned to specific bonds within a molecular structure. The corresponding spectrum makes an allocation much easier than in UV–Vis spectroscopy. The nature of interaction between incident light and the sample differs. Absorptive MIR spectroscopy shows vibrations with alterations in their dipole moment. On the other hand, in Raman spectroscopy the inelastic light scattering of a laser beam interacts with vibrations with temporal changes in polarization. This evolves partially complementary information. For instance, Raman spectroscopy is the first choice for identifying compounds in aqueous solution, since water exhibits strong and broad MIR absorption bands that might overlap with characteristic absorption bands of analytes dissolved in water. Symmetrical, non-polar bonds can be only observed with Raman spectroscopy. In contrast, asymmetric polar bonds such as carbonyl bonds are easily detected by MIR spectroscopy.

NIR spectroscopy is dominated by overtones and combination vibrations. Because of the wavelength position of the corresponding fundamental vibration, mainly

C−H, O−H and N−H overtones can be observed. Strong overlaps of different absorption bands are usually too sophisticated for univariate quantification. Instead, chemometric calibration is necessary in most cases.

6.2.2
Integration of Spectroscopic Techniques into a Microchemical Process

Of course, there are many possibilities for integrating spectroscopic cells into a microreaction system. Because of the small cross-sections within the microfluidic devices, typically no bypass is necessary for such adaptation. Spectroscopic process monitoring can be either realized subsequent to the microreactor setup or directly within the microreactor. As an example, Figure 6.1 shows a pragmatic and flexible setup that allows spectroscopic in-line analysis of microreaction processes. This setup was designed for monitoring parameter screenings and allows adaptation of one or more of the above-mentioned spectroscopic methods. Miniaturized optical

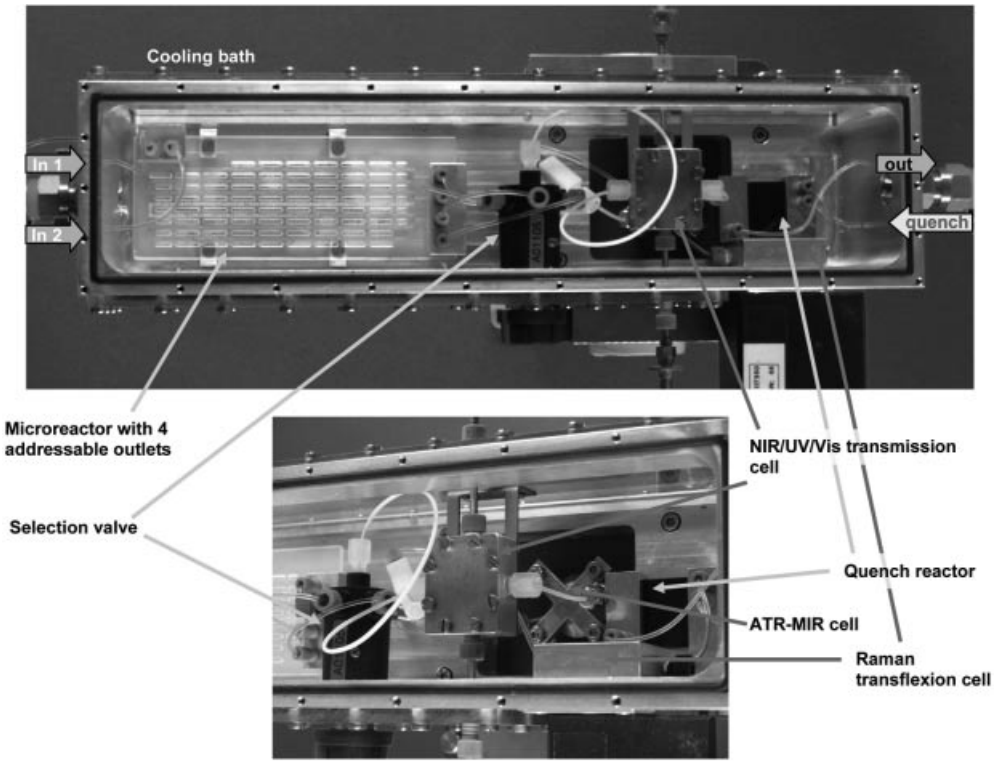


Figure 6.1 Setup of a microreaction screening plant with integrated spectroscopic process analysis. The total microreaction setup can be flooded with a cooling or heating agent to adjust the desired process temperature. External pumps control flow rate and stoichiometry of the reagent solutions. A selection valve adjusts the internal reaction volume of the microreactor (made of glass) with multiple outlets. In addition, up to three optical flow-through cells can be subsequently interconnected, followed by a quench reactor to terminate chemical reaction.

flow-through cells were adapted and connected to the outlet of a microreactor. Which spectroscopic technique is connected depends on the chemical system considered and the type of information needed. If necessary, different spectroscopic techniques can be simultaneously connected and thus directly compared with each other to identify the most appropriate one for a certain process [10]. UV–Vis, NIR and Raman spectrometers are usually connected via fiber optics and probes. For MIR spectroscopy, special miniaturized attenuated total reflection (ATR) flow-through cells turned out to be the best option to monitor liquid-phase reactions. They are connected to the MIR spectrometer via open optical paths. However, recently also optical MIR fibers made of chalcogenides [11] and silver halides [12] have become commercially available. Although they are currently fairly expensive and limited to a length of about 1.5 m, MIR fiber optics might become an interesting option in the near future to connect MIR spectrometers with optical cells or probes.

6.3 Data Generation Using Optical In-line Spectroscopy

6.3.1 Non-concentration-based Information

Within a microchemical system, process parameters such as flow rate, stoichiometry or temperature can be changed and adjusted very quickly. Moreover, if the plant is set up adequately, hold-up and residence times can also be changed during experiments (Figure 6.1). This advantage allows the generation and analysis of several tens of different process conditions per day.

In addition to the capability of identifying and quantifying individual analytes instantaneously within a reaction mixture, non-destructive in-line analytical techniques provide additional information on physico-chemical properties that cannot be obtained by off-line techniques. Non-destructive in-line techniques allow the analysis of existing flow regimes and their influence on the product distribution to provide an appropriate engineering concept. For example, fast spectroscopic techniques reveal temporal macroscopic inhomogeneities in density or analyte distribution within a microreaction setup. Pump pulsation, insufficient mixing or thermal instabilities cause such inhomogeneities, resulting in deviations from the desired steady-state conditions. These indications are very important for process optimization, including the design of the microreactor setup, since suppression of flow inhomogeneities might be decisive for the performance and safety of chemical processes.

In heterogeneous liquid–liquid-phase processes, segmented plug flow (also called slug flow) is often a good choice for optimization of both reactions [13] and extractions. The plugs undergo effective internal circulations within each segment for increasing interfacial mass transfer (Figure 6.2). An additional positive side-effect is the much more defined residence time compared with laminar flow distribution, which highlights one of the major advantages of continuous microchemical processing.

For maximum mass transfer in a segmented plug flow, the segment length has to be as small as possible. For an existing microreaction setup, mainly the flow rate

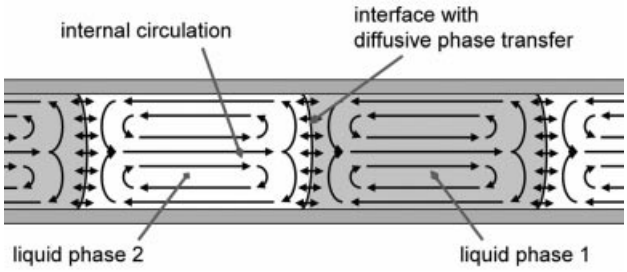


Figure 6.2 Scheme of internal circulations within a slug flow [13].

influences this length. The plugs (or slugs) are usually investigated by using photographic or video camera systems which, for instance, provide detailed information on contact angles and shapes of segments [14]. However, for fast screening purposes, fast spectroscopic methods can be used as an alternative. As an example, Figure 6.3a shows time-resolved spectra of a segmented plug flow in a micromixer–capillary setup obtained by fast NIR spectroscopy using acousto-optic tunable filters (AOTF). The different phases of the plug flow can be easily distinguished by the consecutive minima and maxima along the acquisition time. The corresponding time-resolved changes of the NIR signal at 1900 nm obtained at different flow rates are illustrated in Figure 6.3b. The segment length and its standard deviation can be easily estimated by the time-resolved peak distances at known flow rate and channel geometry. With increasing flow rate, a decrease in segment length and finally the breakdown of the segmented plug flow regime are observed.

In addition to investigations of fluid dynamics, the monitoring of chemical reactions is the major driving force for the integration of in-line spectroscopy. For example, Boskovic's group were among the first to use spatially resolved MIR spectroscopy for the real-time monitoring of specific nitration reactions in microchannels to reveal the chemical pathways and mechanisms of these fast processes [15]. The authors took advantage of the transparency of silicon microreactors in the MIR spectral range to avoid using any additional optical flow-through cell [16]. By focusing the IR beam of an FTIR microscope on different positions along the microchannels of the reactor, series of transmission spectra were measured, describing the progress of the reaction. This technique allowed the monitoring of the completeness of conversion and helped in identifying intermediates of the reaction. Moreover, it was also possible to examine inhomogeneities in flow distribution by comparing the progress of the reaction within the different parallel microchannels of the silicon reactor. Based on such information, a redesign of the microreactor was initiated that resulted in improved mixing and residence time distribution properties [17].

6.3.2
In-line Quantification in Microchemical Processes

An intrinsic need during process optimization is to know the concentrations of species of interest in a reaction mixture, since conversion, selectivity and yield

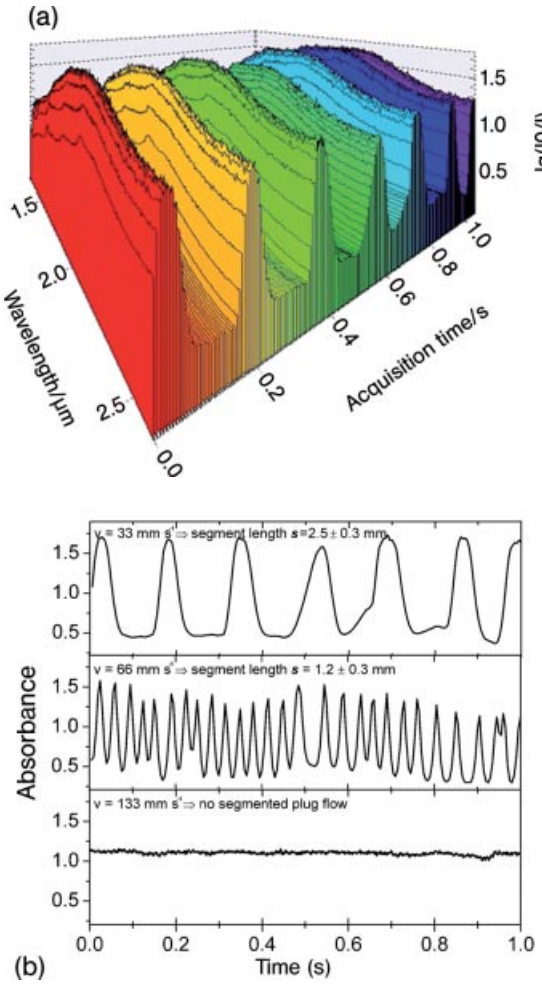


Figure 6.3 Monitoring of a segmented plug-flow reaction in a micromixer–capillary setup (phase 1, toluene; phase 2, mixed acid; capillary i.d., 0.8 mm). (a) Time-resolved NIR spectra at a total flow rate of 33 mm s^{-1}. (b) NIR signal at 1900 nm at different flow rates [10]. Flow rates and segment lengths (including standard deviation) are given in the graphs.

are classical target parameters of optimization procedures. Consequently, quantitative or at least semiquantitative data acquisition is compulsory. However, the huge number of samples that can be produced in a continuous microreaction process within a short period might shift the bottleneck of data acquisition from experimental capacities to analytical capacities, in particular if off-line analytics are applied. Especially separation techniques such as liquid chromatography (LC) or gas chromatography (GC) can be more time consuming than the process optimization procedure itself. Quantitative in-line analysis has the huge

potential to overcome these restrictions or at least decrease the necessity for using separation techniques.

6.3.2.1 Classical (Univariate) Quantification

The simplest form of parameter variation during a continuous microreaction process is the change of reaction time at constant temperature. As an example, Figure 6.4 shows in-line Raman spectra recorded during a polymerization reaction in a microreactor to form a semiconducting polymer for OLED (organic light-emitting diode) applications. The Raman peak detected at 1585 cm^{-1} is allocated to the formation of a highly symmetric C−C double bond in the final polymer. The real-time monitoring of this Raman band allows the determination of the appropriate reaction time for a given process temperature.

In this example, the information about concentration changes is readily available from the measured Raman spectra, since no overlaps between Raman bands of the investigated analytes and other species of the reaction mixture (e.g. solvent) occur. In case of more complex and partly overlapping Raman spectra, multivariate quantification procedures are required as described below.

In the literature, only a few examples of a univariate calibration and analysis applied to a microreaction process can be found. For example, Lu *et al*. described the UV spectroscopic monitoring of the photochemical reaction of benzophenone with 2-propanol to form benzopinacol [18]. In this case, the analytical system was calibrated using an *external non-reactive* standard. A peak decrease between 320

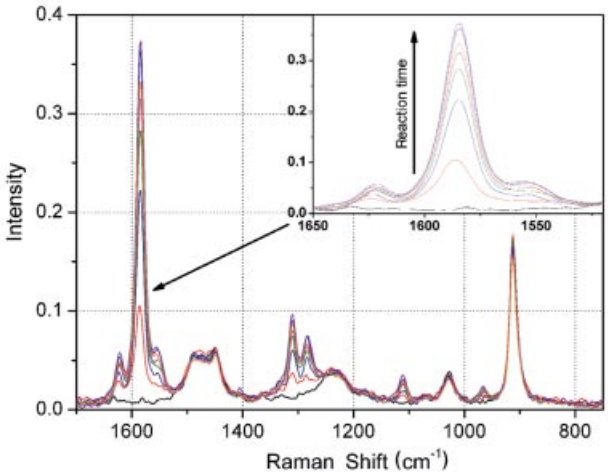


Figure 6.4 Raman-spectroscopic monitoring of a polymerization reaction conducted in a microreactor to form a semiconducting polymer on basis of a polyphenylene–vinylene structure for OLED applications. The C−C double bond at 1580 cm^{-1} that is formed during reaction progress undergoes a strong increase in intensity that allows the determination of the macroscopic reaction rate.

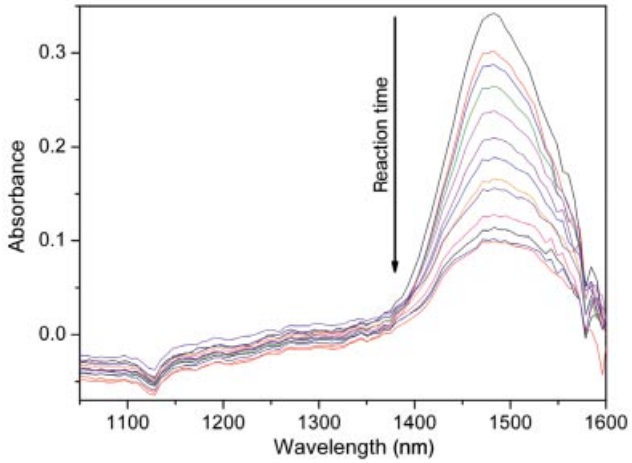


Figure 6.5 Time-resolved NIR spectra of a nitration reaction using mixed acid as nitrating agent [17]. The peak decrease refers to the HNO_3 consumption (background spectrum comprised starting material and H_2SO_4).

and 400 nm was mainly attributed to the starting material benzophenone. The trend was found to be in agreement with results obtained from off-line analysis.

Another example is the NIR spectroscopic examination of processes by quantitatively tracing the first OH overtone vibration at 1400–1600 nm. In this way, aromatic nitration reactions can be monitored by the consumption of HNO_3 [17] (Figure 6.5). On the other hand, condensation reactions generate the detached OH band in NIR spectra that can be clearly assigned to the formation of water.

Although the described spectroscopic methods for quantitative in-line analysis can in principle be applied to all kinds of screening experiments that comprise systematic variations of process parameters, this approach has to be handled with care since some of the parameters have an undesired non-linear effect on a spectrum. For instance, in NIR spectroscopy, an increase in temperature can cause band shifts and influence the peak height. Variations in stoichiometry, which can be easily achieved in a microreaction process by changing the reactant flows, have a direct impact on the analyzed compound concentration. However, such modifications can be calculated subsequent to the reaction, if concentrations are in the linear detection range or the detection range was calibrated in advance.

For example, Leung *et al.* describe investigations of the homogenous catalytic conversion of 2-propanol to acetone that was carried out with a Raman microscope [19]. Skeletal stretching bands of the alcohol (820 cm^{-1}) and acetone (780 cm^{-1}) were calibrated using external standards and were then quantified in the process. In addition to residence time, the stoichiometric ratio between the catalytic system and 2-propanol was changed and successfully quantified by Raman microscopy. It is interesting that in this study, the average residence time of the reaction mixture within the microreactor (1.4–4.5 s) was much shorter than the data

acquisition time (15 s Raman integration time). An appropriate Raman spectroscopic analysis of such fast processes can be only achieved by exploiting the spatially resolved steady-state conditions as can be realized in continuous microreaction processes. Therefore, the Raman spectroscopic analysis of very fast processes in batch processes is limited, since time-dependent concentration changes are much faster than measurement times. Consequently, in batch processes it is decisive for the quality of the analytical results that the measurement rate is significantly faster than the reaction rate.

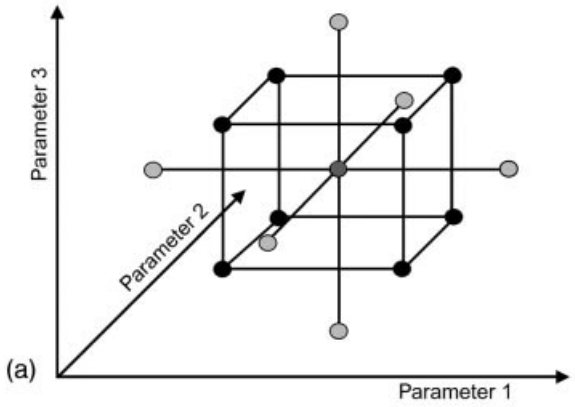
6.3.2.2 **Multivariate Quantification of Complex Reaction Mixtures**

The more complex the reaction mixture and the number of investigated process parameters, the more sophisticated is the extraction of concentration information from measured in-line spectra. There are mainly three reasons that prevent the application of univariate calibration procedures in such cases:

- *Band superposition:* peak overlaps may enforce a mathematical deconvolution of the spectrum to ensure an unambiguous allocation of constituents that contribute to the band superposition. Such mathematical deconvolution is in particular challenging if intermediates only temporarily contribute to peak overlaps.
- *Non-linear effects because of parameter variations:* as mentioned above, variation of process parameters such as temperature enhances the difficulty of setting up an adequate univariate calibration due to band broadening or shifts.
- *Non-linear behavior of the concentration signal:* all spectroscopic methods have a linear detection range. In UV–Vis, NIR and IR spectroscopy, the concentration of an analyte is proportional to its *absorbance* (according to the Lambert–Beer law), whereas in Raman spectroscopy analyte concentration is correlated with *signal intensity* (and not absorbance). The linearity of the detectors of spectrometers is only valid for small concentrations, when interactions among the molecules can be neglected. Moreover, scattering effects in liquid–solid systems can also drastically affect the linearity of the response signal in all spectroscopic techniques.

In all three cases, modern chemometric methods such as partial least-squares (PLS) regression are essential to achieve fast and reliable calibration for quantitative

Figure 6.6 Schematic procedure of a multivariate calibration. Data are taken from the NIR spectroscopic monitoring of the nitration of toluene conducted in microreactors using pure nitric acid as nitrating agent [10]. Note that model optimization is an iterative approach that requires the multiple application of steps (c) and (d). (a) Definition of an experimental design within the investigated parameter space. Here, a central composite plan is presented. (b) Experiments in accordance with the design: spectrum generation in a flow-through cell and subsequent off-line analysis of samples for concentration determination. (c) Spectrum pretreatment (here: first derivative) and selection of wavelength range (hatched part) for model optimization. (d) Multivariate modeling: plot of predicted concentration of an analyte (by the model) versus measured concentration (by off-line-analysis) for estimating quality of calibration. A validation test set delivers quality indicators such as RMSEP and correlation (here: 4-nitrotoluene)


Parameter 3
Parameter 2
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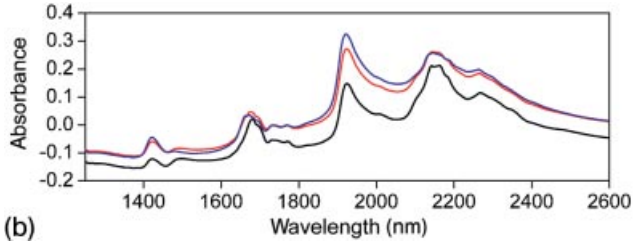
(a)


Absorbance
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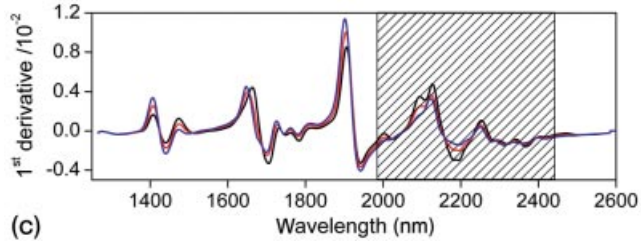
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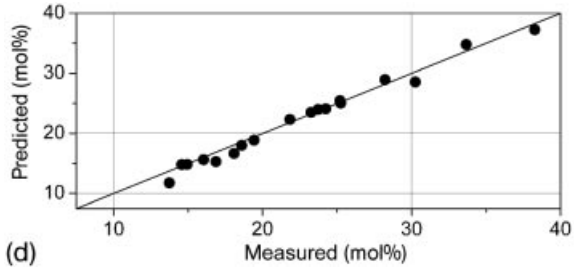
Predicted (mol%)
40
30
20
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10
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30
40
Measured (mol%)
(d)

in-line analysis [20]. Chemometric methods allow easy integration of interfering non-linear spectrum changes that are not caused by changing concentrations of the considered analytes. However, chemometric methods also require representative samples for quantitative calibration (and for validation afterwards). For this purpose, the reaction mixture itself serves as the calibration standard: by employing a quench reactor subsequent to the flow-through cell of the microreaction process, a well-defined termination of the reaction can be achieved (thermal or chemical quench). A sample of such a quenched reaction mixture representing a "snap-shot" of certain process conditions is analyzed off-line with respect to all compounds of interest (e.g. by using HPLC or GC) while a spectrum of this "snap-shot" was measured in-line. The analyte concentrations that are determined off-line in several quenched samples are then used for calibration and validation of the chemometric quantification model. Non-calibrated compounds in the spectrum will be neglected by the model, even if they superpose bands or peaks of the target compounds.

Different calibration standards (quenched reaction mixtures) are obtained by deliberately changing the process parameters of interest. In that context, it is important to note that all process parameters that physically influence the spectrum have to be considered within the calibration procedure. This is decisive for a precise calibration model. If certain changes in the spectrum of a reaction mixture cannot be described by the chemometric model, the quality of predicted values will suffer.

A minimum number of samples with maximum information content can be selected by using methods of experimental design [design of experiments (DoE)] [21]. The principle approach for a central composite design is shown in Figure 6.6a. It presents a parameter space with three different parameters (e.g. stoichiometry, temperature and the total volumetric flow) that might be changed during a microreaction process. Each dot represents one experiment with the corresponding parameters.

The spectra from the calibration set (Figure 6.6b) and the corresponding concentration values determined by off-line-analysis are employed for PLS regression. The underlying algorithm provides a spectral deconvolution into orthogonal factors that are called principal components. The number of principal components corresponds to the number of independent linear changes within the spectra that are necessary for a successful calibration [20]. An additional data pretreatment can improve the calibration. In Figure 6.6c, the generation of the first derivative of the spectra according to the Savitzky–Golay algorithm [22] was applied, using a second-order polynomial.

Finally, a validation set with known concentrations has to be prepared that represents the investigated parameter field. It allows the final evaluation of the calibration quality described by values such as the correlation of predicted and measured concentrations and the root mean square error of prediction (RMSEP), which is comparable to the standard deviation of the predicted values (see Figure 6.6d) [20]. Subsequent to this calibration and validation procedure, a real-time spectroscopic measurement of analyte concentrations within complex reaction mixtures generated in a microreaction process can be accomplished.

6.4 Conclusions

In the last decade, microreaction technology has become a widely accepted process tool for screening, analyzing and optimizing chemical reactions. Furthermore, recent developments show promising applications of microreaction technology for production purposes. In both cases, the integration of analytical methods has the potential to increase the process efficiency, giving access to a broad range of information, some only available by using non-invasive in-line measurement techniques. This includes physical parameters such as the flow behavior and flow distribution within a microreactor plant or the identification of reactive species. Both issues are decisive for an appropriate design of the microchemical process to achieve the best performance.

Moreover, the use of in-line spectroscopic techniques in microchemical processes allows real-time monitoring of analytes and their concentrations in real reaction mixtures. Depending on the characteristics of the chemical process and the objectives of in-line analytical investigations, appropriate calibration methods and procedures might be required.

In particular, if complex reaction mixtures have to be analyzed quantitatively in real time, time-consuming calibration and validation procedures have to be considered. Such sophisticated methods might be mainly the choice in cases of quality and process control during production, but also for long-term in-depth analysis in process optimization studies. However, recent progress in chemometric analysis might lessen this drawback in the future: Modern techniques such as multivariate curve resolution (MCR) promise quantitative determination without any calibration procedure in the near future [23, 24].

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