Part One
Process Control and Quality Assurance

Handbook of Biophotonics. Vol.3: Photonics in Pharmaceutics, Bioanalysis and Environmental Research, First Edition.
Edited by Jürgen Popp, Valery V. Tuchin, Arthur Chiou, and Stefan Heinemann

1
Industrial Perspectives

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1.1
Introduction and Definitions

1.1.1
Introduction

The concept of Quality by Design (QbD) is based on the evident fact that a high level of quality of intermediate or final products cannot be achieved by testing the products but needs to be implemented into the products by intelligently designing the whole manufacturing process. To be able to do so, a comprehensive understanding of the process on a causal basis is required. Only such an understanding ensures reliably defined and consistent quality levels by operating stable and robust processes and, for instance, in the case of pharmaceutical products, allows real-time release of the manufactured goods. The technology that enables translation of the concept QbD into industrial reality is process analytical technology (PAT). PAT is generally considered a science-based and risk-based approach for the analysis and improved control of production processes [1]. Although initially conceived by the FDA for application in the pharmaceutical sector, the PAT initiative continues to grow in importance also for related industries in the applied life sciences, for example, biotechnology, the food industry, as well as the chemical industry [2].

1.1.2
Historical Aspects

In the course of the past decades, the industrial landscape has undergone many changes which were mainly dominated by the shift from a supplier-dominated market to a customer-dominated market (Figure 1.1, [3]). Due to the rebuilding after the Second World War, in the 1950s, the overall product demand exceeded the general capacity supplied by industry. Hence, the quality of a product was mainly defined by the producer's view of what quality was (compare the partial analytical understanding of quality) [4]. This situation has changed dramatically since then. Today the industry faces a market situation that is often characterized by an intense

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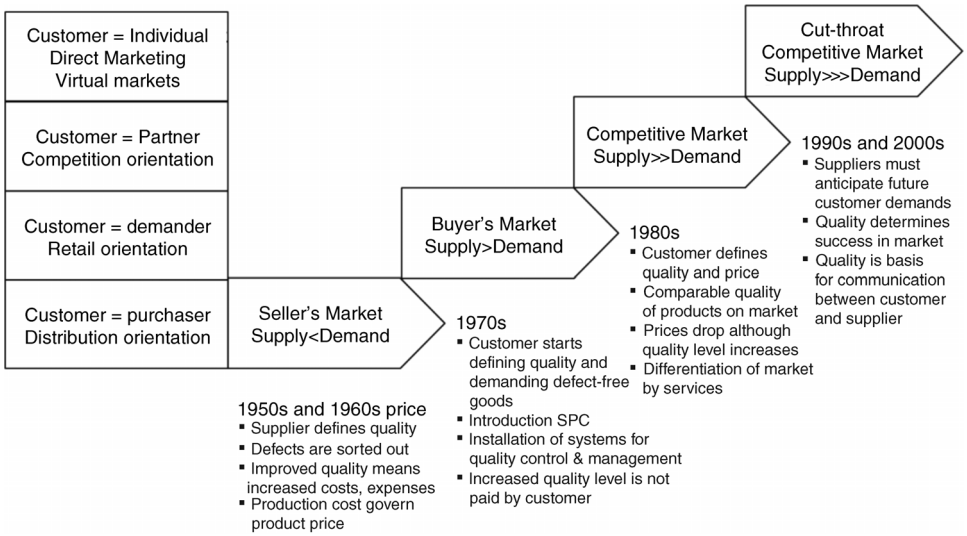


Figure 1.1 Changes in market situation during the past decades and concomitant adaptation of quality concepts (modified after [3]).

cut-throat competition in many branches, by ever increasing product complexity and diversity and by growing customer awareness of high product quality and functionality. In addition, more stringent legislative and regulatory measures imposed by society comprise an increasingly powerful driving force towards comprehensive public health and environmental compatibility of industrial processes. In contrast to the earlier years of industrialization, producing enterprises nowadays can no longer stratify their market position by increasing the mass-per-hour throughput of a certain product. Today, the sustainable success of an industrial company depends more critically than ever on the cost-effective realization of customer-tailored products of high quality that flexibly meet the rapidly changing end-customer's demands.

Reflecting this overall trend towards an increased focus on custom-made quality, increasingly sophisticated and holistic quality management systems have been developed over the years (Figure 1.2), ranging from simple inspection of the finished parts for defects and elimination of inferior ones, over implementing increasingly complex quality systems to avoid the production of any defective parts during manufacturing, to the modern views of process oriented, integrated and comprehensive total quality management systems. It is in this context that the modern concepts of PATs and QbD have to be reviewed. Before this, some general definitions and remarks on quality and process control are given.

1.1.3
Definition of Quality: Product Functionality

Quality may be best defined as product functionality [6]. Several levels of functionality can be identified (see Table 1.1) that are related to the various contexts a product and

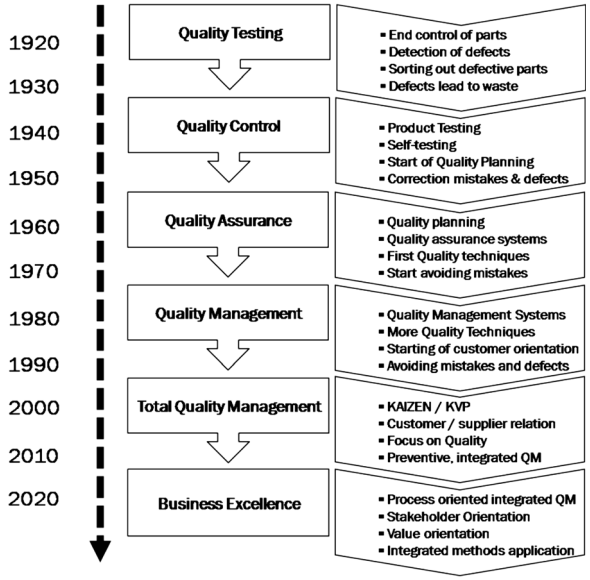


Figure 1.2 Historical development of important quality concepts and their major elements (modified after [5]).

Table 1.1 The various levels of functionality [6].

Functionality level	Description	Examples
Fundamental functionality	Basic properties based on the chemical composition and morphological constitution of the material	Is the material as it should be? Content of ingredients, particle size distribution, distribution of active compounds/traces/ dopants within material, and so on
Technical functionality	Behavior during the production process	Is the product processable? Flow behavior, mixing properties, purification and down-streaming properties, and so on
Technological functionality Value-oriented functionality	Required performance profile for the intended use Cost : benefit ratio	Is the product usable? Hardness, strength, efficacy, durability, Is the tailored quality level appropriate? Displayed product features versus price
Sensory functionality	Appearance and design	Is the product appealing? Haptic behavior, product smell, visual appearance, and so on

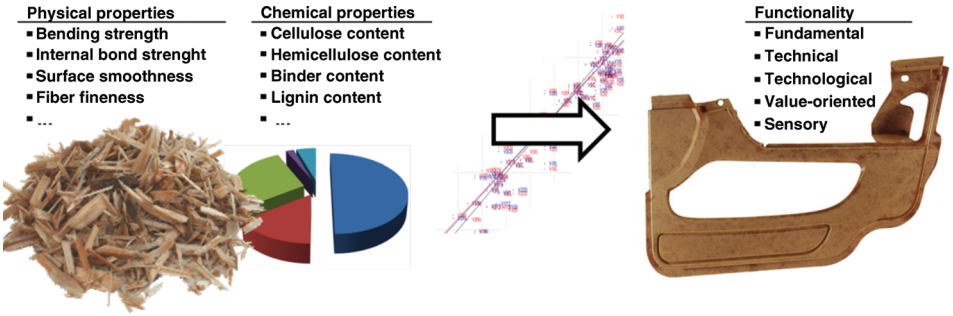


Figure 1.3 Relationship between measurement and product functionality.

its intermediates experience throughout the whole life cycle from manufacturing to the end-use and its disposal. Besides its fundamental functionality, which relates to the basic physical and chemical properties, such as composition and components distribution, surface properties or morphology of a product, the product must also be processable during manufacturing (technical functionality) and must fulfill the customer's requirements during the end-use (technological functionality). Moreover, the extent to which certain quality levels are realized in the product (value-oriented functionality) and its design-related properties (sensory functionality) are also important aspects. In the ideal case, single measured values from various methods are combined and mathematically related to all these different functionality levels (Figure 1.3).

According to the Kano model [7], a product complies with the customer's requirements and expectations when it displays basic, performance and excitement functionalities [8]. All performance characteristics of a group of objects or services intended to successfully populate a certain product niche will steadily have to be improved, expanded and further developed with time, since the customer will get used to the features and functions. This provides the driving force for continuous product development and product innovation. Due to cost issues and a desirably short time-to-market of novel products, only companies that are able to handle very short innovation cycles will sustain economic success. Knowledge-based production, based on a QbD approach and realized by process analytics, is the key element in achieving such short innovation cycles. Furthermore, it allows flexible response to sudden changes in customer's expectations, since the processes to translate the quality characteristics into product features are causally understood.

In this context, two main aspects need to be considered when introducing process analytical tools, for example, on-line spectroscopy, into manufacturing: *quality monitoring* and *product functionality design* [6]. Usually, the identification and quantification of a direct relation between, for instance, the measured spectral information and a target compound like a pharmaceutical ingredient is attempted. In many cases, univariate target responses, such as concentration, purity, or extent of conversion, and so on are determined and compared with standard values. Thereby, deviations of characteristic process parameters may be determined in real-time and the quality of

the manufacturing process may be monitored and controlled. Due to the recent developments of stable on-line and in-line instrumentation, in combination with complex chemometric toolboxes, a robust calibration of such relations is possible and, therefore, applications of process analytics in industry are numerous. In most cases, off-line measurements can be directly substituted by on-line spectroscopy, with the advantage of a possible 100% quality control of a specific response.

However, while process analytics is already widely recognized as a powerful tool to perform such *quality monitoring*, the full potential of this technology is by far not exhausted; process analysis can be exploited to an even much greater extent with economical advantage when it is embedded in a philosophy of continuous process improvement and *product functionality design*. Currently, process analytical data obtained from measurements on intermediate product stages during the running manufacturing process are only very rarely related to the performance of the final product or to the final application properties of the product. However, it is possible to relate process analytical information even to product functionality when a consequent transition from considering univariate data (single parameters and responses) to multivariate data analysis (multiple parameters and responses) is performed. Product functionality may be defined as the fundamental chemical and morphological properties of the material, by its performance through manufacturing, by the technical properties for the final application, and certainly also by its cost/performance ratio. If objectively classifying data for these definitions exist, a direct correlation to, for example, the spectral information in the case of on-line spectroscopy is possible. The exact nature of the individual signature of a spectrum (the "*spectral fingerprint*") is always dominated by the morphology and chemistry of the substrate, due to its substance specific absorbance and scattering behavior. The relative contributions of these two components to the measured spectrum depend on the wavelength of the interaction, on the angle of illumination of the substrate, on the angle of detection, on the difference in refractive indices, and on the particle size and particle distribution. In Figure 1.4 this is illustrated using the example of tablet spectroscopy [9].

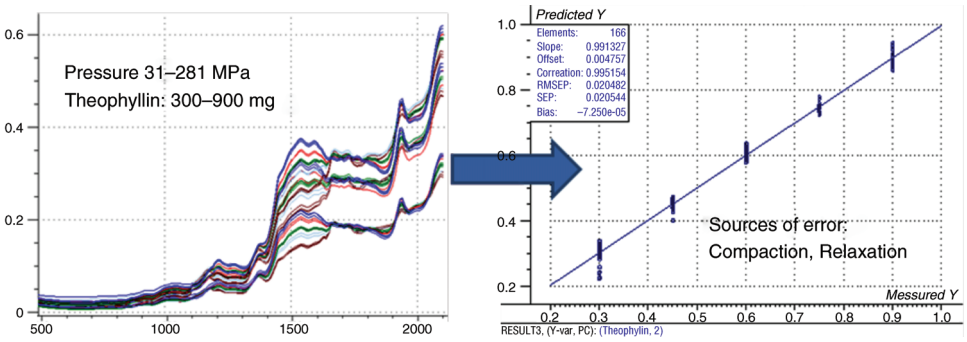


Figure 1.4 The information contained in wavelength-dependent scattering and absorption spectra of tablet samples compacted at different pressures (ranging from 31 to 281 MPa) and containing different amounts of theophyllin can be used to model the theophyllin content [9].

This multivariate information can be used not only to calculate the dependence of a single target (*quality monitoring*), but also allows full and overall classification of the sample quality (*functionality design*). This is especially true for the characterization of solids and surfaces by means of diffuse reflectance spectroscopy. Within the concept of QbD/PAT, a knowledge-based manufacturing is attempted which relies heavily on the combination of various sources of information using chemometric methods like principal component analysis, PLS, multivariate curve resolution, or other multivariate calibration methods.

1.1.4 Quality Control

The term *quality control* may generally be defined as a system that maintains a desired level of quality [10]. In general, this may be accomplished by comparing a specific quality characteristic of some product or service with a reference, and if deviations from the desired state are detected, taking remedial action to reinstate the targeted quality level. Similarly, *process control* may be defined as appropriate measures to readjust the state of a process upon some observed undesired deviation. *Process analytics* provides the required information on the state of the process. While *process analytics* deals with the actual determination of specific data using process analytical devices, like HPLC, optical spectroscopy or other sensors, *process analytical technology* is a system for designing, analyzing and controlling manufacturing by timely measurements [1] (see below), already with quality determination in mind. *Process analysis* is the comprehensive analysis of the industrial process including every single activity involved in the manufacturing of the product. Thereby, all material and virtual flows are considered. Historically, the *PAT initiative* roots in a comprehensive approach to realizing *process analysis* on an instrumental basis. PAT is the essential tool to realize the concept of *QbD*, which has recently been further developed to the even more comprehensive approach of product quality life-cycle implementation (PQLI) [11, 12].

Essentially, quality control is accomplished by *off-line quality control* procedures, *statistical process control* and, to a lesser degree, by *acceptance sampling plans*. *Off-line quality control* involves selecting and defining controllable product and process parameters in such a way that deviations between process output and a standard will be minimized [10]. A typical tool for such a product or process design is the statistical experimental design approach or design of experiment (DoE). Quality is here basically defined "off-line" before the process has actually been implemented or started. *Statistical process control* (SPC) in contrast compares the results or output of a process with the designated reference states and measures are taken when deviations from a desired condition of a process are statistically significant. When the process is poorly designed (by inappropriate off-line quality control measures, that is, unsuitable or sub-optimal processes) these deviations may be large and cannot be compensated for by statistical process control. Hence, it is obvious that off-line quality control by well-designed processes which are based on a thorough understanding of the effects of the involved process factors on the critical quality features of the product

will govern the achievable product performance, or in other words: quality cannot be tested into products afterwards.

1.1.5
Quality Assurance

Quality assurance relates to a formal system that ensures that all procedures that have been designed and planned to produce quality of a certain level are appropriately followed. Hence, quality assurance acts on a meta-level and continually surveys the effectiveness of the quality philosophy of a company. Internal and external audits, standardized procedures and comprehensive documentation systems (traceability) are important tools to accomplish this "watchdog" function within the company. Strict process descriptions determining every single step required during manufacturing a product, including the required appraisal procedures, may be defined, and deviations from these fixed procedures may be indicative of potential deteriorations in quality; instruments like the Good Manufacturing Practice approach or ISO certifications are typical for quality assurance on a highly sophisticated level. However, defined procedures and certification alone do not necessarily lead to improved performance or functionality of a product; obeying agreed-on procedures merely guarantees conformance within a specifically designed process. Pre-defined and fixed processes that are certified and commissioned by the regulatory authorities, like for instance the manufacturing process of a specific drug by a pharmaceutical company which is accepted and granted by authorities like the Food and Drug Administration (FDA) may even prove to be inflexible, sub-optimal and difficult to develop further. Since every small deviation from the standard routine processing is considered a potential quality risk and, especially in the case of pharmaceuticals or biologicals, may comprise a potential health hazard, all such deviations are required to be communicated to the authorities. Process improvements or further adaptations that usually require significant redefinition of process parameter values need renewed approval by the authorities, which in most cases is time and cost intensive. Thus, in a sense, quality assurance may even be counterproductive to process improvement and impede the establishment of higher quality levels in products. To overcome these limitations of current quality assurance policies, during the past years, the FDA has promoted the PAT initiative which, in a similar form, is also supported by the European Medicine Agency (EMA).

1.2
Management and Strategy

1.2.1
PAT Initiative

The major incentive behind the *PAT initiative* of the FDA is defined in the FDA-Guidance "PAT – a Framework for Innovative Pharmaceutical Development,

Manufacturing and Quality Assurance" [1]: "PAT is a system for designing, analyzing, and controlling manufacturing through timely measurements (i.e., during processing) or critical quality and performance attributes of raw and in-process materials and processes, with the goal of ensuring final product quality. It is important to note that the term *analytical* in PAT is viewed broadly to include chemical, physical, microbiological, mathematical, and risk analysis conducted in an integrated manner." Within the PAT initiative, the manufacturers of pharmaceutical compounds are motivated to undergo a transition from the currently used strategy of testing random samples at the end of the pipeline for their compliance and otherwise strictly sticking to approved routine procedures, towards a causal understanding of the process by means of process-accompanying and process-controlling measurements and tests. This PAT recommendation is valid also for other branches of industry, such as the food industry or biotechnology. By using powerful process analysis in the sense of PAT, manufacturing processes may be controlled and directed towards the desired levels of quality; moreover, PAT also contributes to the resource-efficiency of the production process by, for instance, minimizing the emission of carbon dioxide or reducing the energy consumption. Ideally, a 100% control of the manufactured goods is accomplished by using on-line and in-line sensors. It is anticipated that, by an integrative and system-oriented approach based on process analysis and process control, the industry will experience significant competitive advantages in the manufacturing of high-quality, customized products. Explicitly, the following goals are pursued with employment of process analysis and process control tools (PAT):

- Increase in productivity and product yield
- Minimization of energy and resources consumption
- Minimization of expenses for safety issues in the production facility
- Decreased number of customer complaints
- Increased operational flexibility
- Anticipating maintenance and process-integrated self-diagnosis
- 100% constant and certified quality

PAT will increase the production efficiency by

- Deep understanding of the production process
- Integration of quality into process steps
- Reduction of quality overhead costs
- Higher production quality
- Lower production costs
- Self-adjusting production processes

With the implementation of PAT it will be possible to pursue product-functionality design or a quality by design approach from the very beginning of the product conception. PAT targets a comprehensive feed-forward control approach and adaptive process analysis systems. Implementing PAT consequently requires application of the following modules:

1) Risk analysis of the production process (e.g., by failure mode and effects analysis, FMEA)
2) Process analytics (sensors, spectrometers, etc.)
3) Process control systems (SPC, MSPC)
4) Statistical experimental design (DoE)
5) Multivariate data analysis

This PAT toolbox and its interplay is depicted schematically in Figure 1.5.

1.2.2 PAT Toolbox

Hence, in contrast to a widely anticipated false view, PAT is not restricted to single devices for process analysis; PAT covers numerous tools included multivariate statistical methods for data design, data gathering and data analysis, process analytical sensors, control systems in the manufacturing, testing and admitting of products, as well as measures for continuous process improvement and methods of knowledge management. One of the most important groups of on-line sensors is the *spectroscopic sensors*, using the interaction between electromagnetic radiation and matter for material characterization [6]. Another important group of PAT sensors are based on *chromatographic methods* which employ various types of physical-chemical separation principles to physically de-convolute complex reaction mixtures into single components which may subsequently be identified and quantitatively determined [13]. Yet another and completely different group of sensors is the so-called *soft sensors* [14]. The basic principle behind soft sensors is that some material properties

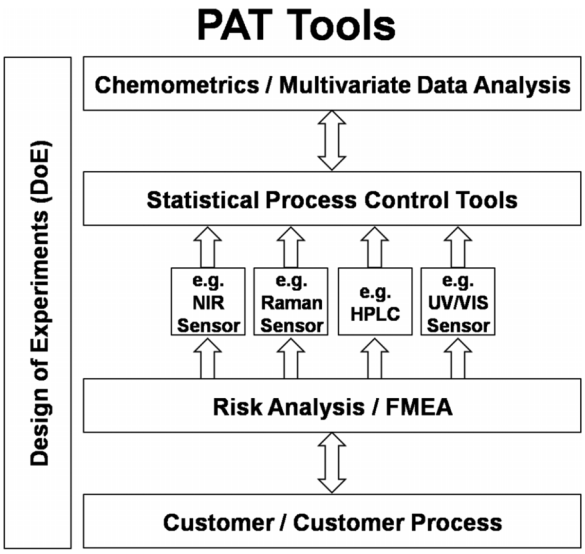


Figure 1.5 The PAT toolbox.

may not be measured directly as physical or chemical material parameters but can only be deduced indirectly by analyzing secondary variables, which in turn can be related to the target property by mathematical models. A very interesting recent account of soft sensors in the PAT context is given in Ref. [14]. With soft sensors, basically two main groups can be distinguished. On the one hand there are purely data driven models which involve no *a priori* knowledge of biological, physical or chemical interrelationships between the various categories of variables. Such models are called *black box models* and have the advantages of requiring no deep process understanding and relative ease of implementation. However, they may over-fit the observed data and be restricted to pure descriptions of the data without yielding true causal relationships which are required for a knowledge-based quality design. Tools often used are artificial neuronal networks, evolutionary algorithms, chemometric models like partial-least squares (PLS), principal component analysis (PCA), principle component regression (PLR), or support vector machines (SVR). *White box models*, in contrast, are based on known physical or chemical relationships based on kinetic or thermodynamic equations. If *a priori* information is integrated into data driven models, so-called *gray box models* are employed [14]. It is evident that numerous mathematical methods and algorithms are also included in PAT and, hence, PAT is not restricted to specific sensors that record specific physical signals. There are numerous requirements from the industrial user for process analytical technologies. The most important ones are summarized in Figure 1.6 [15].

1.2.3 The Concept of Quality by Design (QbD)

The basic concept behind PAT and QbD in the context of regulatory authorities of the pharmaceutical industry has only recently been summarized very concisely by

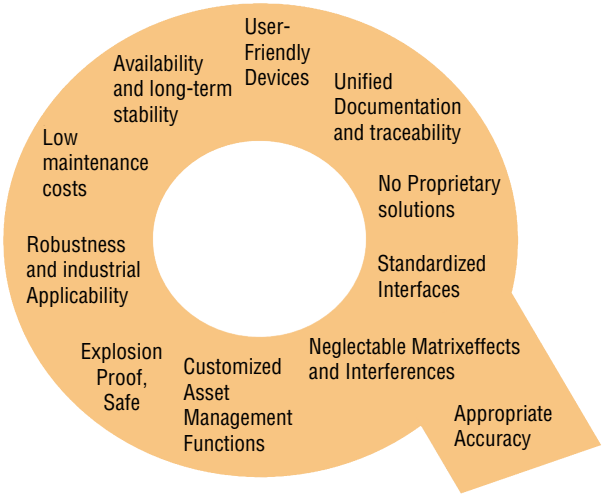


Figure 1.6 User requirements for PAT tools Source: [15].

Schmidt-Bader [16] and in the following paragraphs some of the most relevant aspects from this article will be adapted and summarized. The key aspect with a QbD approach is that quality should not simply be tested at the end after the manufacturing process has finished but should be considered already in the early phase during the conceptual design of the product, and later at all stages of its manufacture. "Quality cannot be tested into products; quality should be built in by design" [1]. In consequence, quality becomes already a matter of product development and, hence, is also strongly dependent on prior research activities into how the desired product features may be realized by industrial processes. The whole processing cycle, ranging from the early developmental stage when the product and its quality features are designed and planned, based on the input from the customers, over the product realization and production phase to its final distribution and end-use is included in such a perspective, and the manufacturer is now in the situation that he needs to demonstrate a causal process understanding throughout the whole cycle, starting from the early phases of product development to the routine production which allows guaranteed compliance with the required critical quality attributes (CQAs) during all steps. This can only be brought about by employing scientific methods. "Using this approach of building quality into products, this guidance highlights the necessity for process understanding and opportunities for improving manufacturing efficiencies through innovation and enhanced scientific communication between manufacturers and the Agency" [1].

All in all, with the PAT initiative the industry faces a shift in paradigm regarding the future of intelligent process and quality control from a *quality by testing* approach towards a *quality by design* approach. This paradigm change offers many opportunities for business excellence. Primary goals in the context of QbD are

- Assurance of a reproducibly high quality of the intermediate and final products
- Reduction of the manufacturing costs
- The promotion and advancement of novel, innovative technologies for quality assurance and process optimization
- The generation of in-depth, causal understanding of manufacturing processes

The transition from QbT to QbD is characterized by numerous significant changes in quality philosophy which are summarized in Figure 1.7 and Table 1.2.

1.2.4 ICH

The concept of QbD as developed by the FDA has been pushed towards realization during the past years mainly by the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) [18]. The motivation behind ICH was to stimulate world-wide a common and more flexible, science-based approach to the admission of pharmaceuticals. The main focus lies in an international consensus between regulatory authorities and industrial companies regarding the quality of pharmaceutical compounds: "Develop a harmonized pharmaceutical quality system applicable across the life

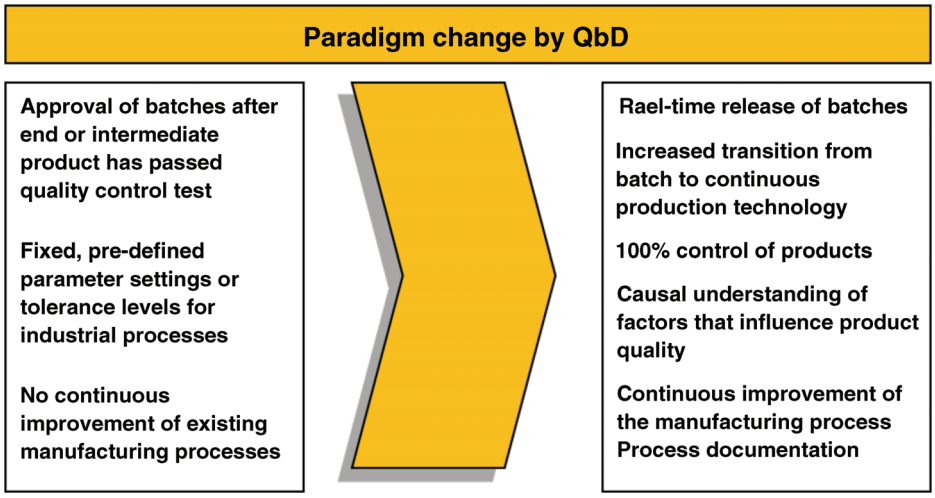


Figure 1.7 Paradigm change by QbD (Courtesy of JH & Partner CATADIA Consulting GmbH, Germany).

cycle of the product emphasizing an integrated approach to risk management and science".

In the ICH Q8 document [19] quality in the context of the QbD concept is defined as follows: "Quality is the suitability of either a drug substance or drug product for its intended use. This term includes such attributes as the identity, strength, and

Table 1.2 Major differences between QbT and QbD (modified after [17]).

	Quality by Testing (QbT)	Quality by Design (QbD)
Development	• Empirical approach • Importance of random findings • Focus on optimization	• Systematic approach • Multivariate strategies • Focus on robustness
Manufacturing	• Fixed • Based on defined specifications	• Variable within design space • Based on knowledge • Supported by robust processes
Process control	• Retrospective analysis • Based on in-process control quality is determined • Data variability is not completely understood • Focus on reproducibility	• Prospective analysis • PAT tools control critical parameters, quality is predicted • Data variability has been subject of research and is completely (causally) understood • Focus on PAT and QbD concepts
Control strategy	• Feed-back control • Control by testing and inspection	• Feed-forward control • Knowledge- and risk-based quality assurance
Product specification	• Acceptance criteria depend on data of specific product charge	• Acceptance criteria depend on end-user benefit

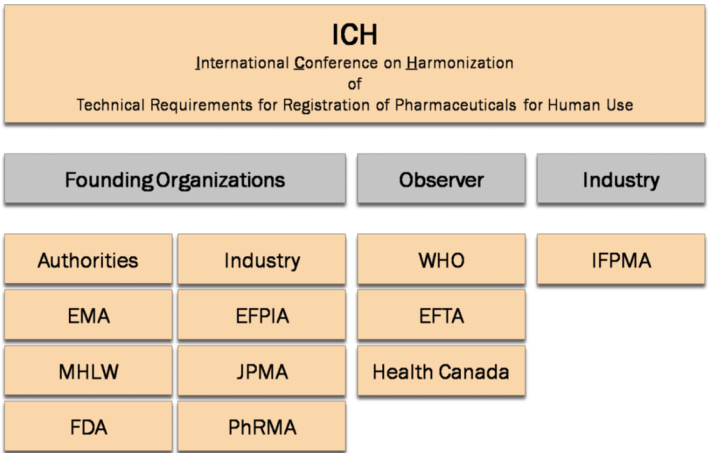


Figure 1.8 The organizational structure of the International Conference of Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) [18].

purity." [19] Besides considering the traditional definition of drug quality in the sense of the law, the ICH definition also includes all processes and parameters that might have an impact on the quality of the drug and, as documented in the ICH Q6 publication [20], ICH clearly assigns full responsibility to the industrial manufacturers to provide the required level of quality of a drug by appropriate measures: "Specifications are critical quality standards that are proposed and justified by the manufacturer and approved by the authorities" [20].

ICH was founded in 1990 by six independent organizations representing the regulatory authorities as well as the industry involved in pharmaceutical research in USA, Europe and Japan (see Figure 1.8).

The organizations directly involved are

- European Medicines Agency (EMEA)
- European Federation of Pharmaceutical Industries and Associations (EFPIA)
- Food and Drug Administration (FDA)
- Pharmaceutical Research and Manufacturers of America (PhRMA)
- Ministry of Health, Labor and Welfare (MHLW)
- Japan Pharmaceutical Manufacturers Association (JPMA)
- International Federation of Pharmaceutical Manufacturers and Associations (IFPMA)

The European regulatory authorities are represented by the Committee for Medicinal Products for Human Use (CHMP) as a subsection of the EMA. The European pharmaceutical market is represented by EFPIA which represents 29 national pharmaceutical associations in Europe and 45 of the most important industrial companies. For the American authorities, the FDA is involved with the Center for Drug Evaluation and Research (CDER) and the Center for Biologics Evaluation and Research (CBER). The researching American pharmaceutical indus-

try is involved with the PhRMA which represents 67 industrial enterprises and 24 research organizations from the US. The Japanese authorities are represented by the Pharmaceuticals and Medical Devices Agency (PDMA) and the National Institute of Health Sciences (NIHS) while the Japanese industry is represented by the JPMA which heads 75 Japanese pharmaceutical companies and 14 national committees. Pharmaceutical companies and associations situated in other countries all over the world, including threshold and developing countries, are represented by the IFPMA, which is a non-profit, non-governmental organization comprising numerous companies involved in pharmaceutical research, biotechnology and vaccine manufacture.

Additionally, three international organizations of observing status are involved in ICH, whose most important role is to mediate between ICH and non-ICH member countries. These three organizations are

- World Health Organization (WHO)
- European Free Trade Association (EFTA)
- Health Canada

1.2.5 The Concept of a Design Space

For an understanding of the strategy behind PAT/QbD, the concept of a design space is of special importance. The ICH Q8 document [19] defines a design space as a multidimensional correlation, that is, the combination and interaction of numerous production factors governing the built-in quality of a (pharmaceutical) product. Within such a design space, the complex interplay between input variables, like properties of raw materials, process parameters, machine properties or user-effects are completely understood on a causal level (see Figure 1.9).

Causal understanding is achieved by identifying and quantifying the effects of critical factors on product quality at any stage of the process by multivariate mathematical models. A design space can be obtained by applying the design of experiments (DoE) approach which has been established as an important tool in quality management since the 1980s [21, 22], for example, in the Six Sigma concept [10, 23].

As pointed out in the ICH Q8 document [19], from a regulatory point of view, a process would be considered as conforming as long as it is carried out within the predefined design space. Since the exact trace within the design space is of no importance, manufacturing a pharmaceutical or any other product becomes more flexible. Currently, even small deviations from pre-defined values of process parameters need to be addressed with the authorities in a time-consuming and cost-intensive procedure. Such deviations from a specific path within the design space would no longer matter as long as a defined level of final quality can still be guaranteed.

As an example to illustrate this shift in process philosophy in the pharmaceutical industry, an industrial process will be discussed schematically. Consider the chemical synthesis of a given compound X. According to conventional philosophy, the

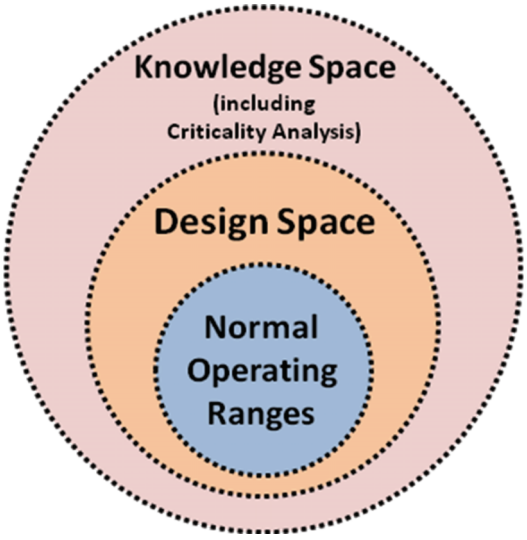


Figure 1.9 Schematic representation of the design space which is based on a knowledge space. The control strategy with a QbD approach is to maintain a process within the design space.

preparation process would have been required to be defined very specifically in terms of production conditions, such as specified reaction temperature, reaction time and, say, catalyst concentration. To conform with the certified procedure all efforts are focused on keeping the process within defined limits. Process analytical technology would be employed to control the parameter settings for temperature and catalyst concentration within agreed narrow boundaries to carry out the process in the predefined way. The target in the traditional philosophy of QbT would be to maintain a process "180 min at 120 °C with 5% of catalyst", all significant deviations therefrom would have to be documented and announced. In a QbD approach, in contrast, the target would be "95% conversion plus defined product specifications" and all feasible combinations of temperature, time and catalyst concentration (= design space) leading to this end would be allowed. Thereby, a quality (and risk) based approach to production is realized and much regulatory effort and certification cost can be avoided.

This offers process analytical technology a decisive role in manufacturing. Process analytical tools would not be used to "measure and control the quality of the product" or intermediate stages thereof, but instead would be employed to determine the current "position of the process in the design space" allowing prediction of the expected degree of quality and adjusting accordingly subsequent process steps to guarantee an agreed final level of quality.

Accordingly, validation of the manufacturing procedure would be focused on the process analytical methods that allow complete control of the design space. Robust process analytical technologies are the key requirement for monitoring and con-

trolling the multivariate design space [16]. Analytically robust methods yield precise and accurate results under modified working conditions. Analytical ruggedness is of importance, too. This means that analytical methods are reproducible under varying analytical circumstances, such as different laboratories, different laboratory personal or different measurement devices. Robustness and reliability of analytical methods as well as their continuous improvement will be of increasing importance for assuring the quality and safety of products. In consequence, industrial processes will become more robust. The robustness of an industrial process may be defined as its tolerance against variability introduced by fluctuations in raw material quality, variations in process and environmental conditions, and variations introduced by equipment (e.g., deterioration with time) and human resources (e.g., habits, moods). With robust processes, companies may be allowed to produce their goods with a higher level of independence at lower cost and still improved guaranteed quality levels.

Currently, manufacturing processes are defined and certified at an early stage when only incomplete information is available on the influence of variations and fluctuations in raw material quality and process parameters. Improvements based on later experience are difficult to implement. Knowledge-based manufacturing allows rapid and continuous adaptation of the process to varying starting conditions and allows cost-effective further development and quality improvement.

A major element within the philosophy of QbD is the exploitation of PAT to accomplish this transition towards a knowledge-based production.

1.2.6 Implications for Other Branches of the Life Sciences

1.2.6.1 General Remarks

Although the basic impetus for pursuing PAT/QbD approaches arises from restrictions imposed by the rigorous legislative and regulatory framework in the context of the good manufacturing policy (GMP) in the pharmaceutical industry, which lead to complicated and cost-intensive approval processes caused by already moderate modifications or even improvements in the production process, the implications of this approach are certainly also of importance for other branches of the life sciences. For example, in biotechnology and the food industry, the total control over the design space in the growth of microorganisms and the harvesting of compounds produced by them has also been targeted recently [24]. Compared to the pharmaceutical industry, however, there are several peculiarities that have to be overcome by these industries, many of which deal with process analytical research questions. Although many of the chemical processes employed in the pharmaceutical industry which are desired to result in well defined compounds of high purity with a very specific biological activity are complex systems governed by numerous factors like concentration, composition, temperature, pH, and so on, in comparison to biotechnological or biosynthetic processes they seem rather simple and well-defined with respect to the possible synthetic pathways encountered.

1.2.6.2 **Biotechnology**

In biotechnology, due to the introduction of living cells or even mixed cultures, another level of complexity is introduced which usually renders classical chemical reaction engineering insufficient to develop a full understanding of the relevant design space. Considering, for instance, recombinant protein expression by genetically modified microorganisms, this typically involves numerous inter- and intracellular interactions, growth and diffusion phenomena, and very complex chemical reaction cascades within the living cells of the microorganisms. Expression of the foreign target compound by the organism has to be balanced with the energy and material demands of the growing microorganism. Intracellular transport phenomena and metabolic processes have to be included in the quantitatively and causally determined design space, as well as external factors like organism selection, fermentation medium development, process parameters, and scale-up effects. Hence, like in conventional chemical or pharmaceutical synthesis, the reactor system may not be treated as a black box; in the case of biotechnology the living microorganism systems need also to be scientifically understood. This imposes great challenges on process analytical technologies and is reflected by numerous recent attempts to monitor in real-time fermentation processes and the cultivation of microorganisms. Although subsequent process steps in biotechnology, such as down-streaming, that is, the purification and enrichment of the desired compounds from the fermentation broth, are also important, the major focus in the application of PAT/QbD concepts in biotechnology clearly lies in the fermentation step. Quality improvement in the course of the down-streaming may be achieved only to a certain degree, and is usually achieved only via a sequence of several process steps and, hence, is rather time-consuming and cost-intensive. Therefore, the development and adaptation of suitable in-line measurement technologies that lead to an improved understanding of microbial fermentation is the most effective and promising way. Again, in comparison to the pharmaceutical industry which was our starting point, specific problem solutions of process analytical tools for biotechnology must, among others, consider that the sterility of the reaction system must be ensured, that many of the targeted analyte species are present only in very small amounts, and that there is usually a large influence of the surrounding medium, which in general is rather complex and not constant with time.

1.2.6.3 **Food Industry**

Another level of complexity is typically added when the food manufacturing industry is considered from a holistic point of view. Here again, purely physical and chemical processes similar to classical chemical and pharmaceutical industries or fermentation processes as in biotechnology may be involved in the manufacturing of food. However, the growth, harvesting and processing of multi-cellular organisms and complex objects has to be taken into consideration, and should actually be included in the causal analysis of the variation pattern observed in food manufacturing. Again, powerful process analytical technologies are required that include measurement and processing of reliable data. However, in the context of

establishing PAT/QbD approaches in the manufacturing industry, the food industry plays a special role, not only because of the complexity of the involved processes but also because it displays a disproportionately low level of automation in comparison to the chemical and pharmaceutical industries or other branches. Hence, the demand for process analytical technologies is especially high. It can be safely assumed that due to the ever increasing cost pressure, to globalization, and to the increasing requirements regarding quality assurance and food safety the food industry will develop to an important emerging market for the automation industry in the coming years. Besides activities directed at the rationalization of not-yet automated processes, two main fields will be of major importance in the food industry, (i) pro-active process and quality management, involving the integration of quality assurance in the production and an improvement in equipment availability; and (ii) the tracking and tracing of goods, that is mainly targeted at an increase in food safety and a reduction in food deterioration caused by microbial decay processes. This implies, in turn, an increasing demand for the engineering and development of appropriate process analytical technologies, suchas, among others, in-line sensors and data extraction tools [25].

1.2.6.4 **Summary and Outlook**

In any industry, process analytical technologies will, hence, gain in importance in the near future. Process analysis as one of the major tools within PAT is concerned with chemical, physical, biological and mathematical techniques and methods for the prompt or real-time acquisition of critical parameters of chemical, physical, biological or environmental processes. The aim of process analysis is to make available relevant information and data required for the optimization and automation of processes (process control) to assure product quality in safe, environmentally compatible and cost-efficient processes [12].

Not only does time-resolved information need to be retrieved and used as a controlling input, but, as becomes most evident from the outlined increase in complexity of the subjects that are dealt with in the life sciences, the retrieved information should also be space-resolved (chemical imaging). While in rather "simple" aqueous reaction systems with quite rapid adjustment of (dynamic) equilibria during chemical synthesis a spatial resolution may not necessarily be required, it is obvious that the distribution of a drug in a pharmaceutical formulation, or the spatial distribution of pathogens on food crops may be of critical importance to the overall quality of the manufactured goods.

The major improvements for industrial processes that are brought about by pursuing a QbD approach with the concomitant rigorous application of PAT tools may be summarized as follows:

- Assurance of reproducibly high quality of intermediates and final products
- Reduction of the manufacturing costs
- Continuous process optimization with respect to an improved exploitation of the employed material and energy resources
- Improved yields of high-quality end-product

- Improved safety and environmental compatibility of the industrial processes
- Stimulation of novel technologies for quality assurance and process optimization
- Generation of a causal understanding of the manufacturing process

1.3
Toolboxes for Process Control and Understanding

1.3.1
Introduction: Causality

Process control and understanding is an important feature for a future knowledge-based manufacturing. Although on-line process control has been well known for the last 20 years, the aspect of PAT in the pharmaceutical industry has become a driving force for recent activities [1]. However, "quality" has different meanings to different companies. For instance, for large companies that produce a standardized material, the major target associated with quality is ensuring close adherence to the defined product specification. In contrast, for smaller companies, "quality" means, in many cases, guaranteeing the flexibility to fit the end-product requirements in relation to rapidly changing market needs. Moreover, nowadays the concept of quality often goes far beyond a specific product but embraces the concepts of plant quality and total quality management (TQM). However, what all views of quality have in common is their dependence on information about the intrinsic properties of a product and knowledge of the relationship between plant parameters and product functionalities.

On-line and inline quality control and process optimization will only be successful when based on appropriate process analysis and process understanding, that is, the analysis of the connection (cause and effect) between process parameters and the quality characteristics of the final product with its specifications. Process analytics in this sense means, therefore, understanding the causal relation between measurement and response. By definition, causality is the strict relationship between an event (the cause) and a second event (the effect), where the second event is a consequence of the first [26]. Very often in chemometrics only descriptive or statistical knowledge is produced which fits the special data set but cannot be used as a general model. Figure 1.10 visualizes the different levels of knowledge for process understanding.

A straightforward "cooking recipe" for knowledge-based production integrates the following procedures (see also Section 1.2.2, Figure 1.5):

Step 1: Detailed analysis of the process and risk assessment
Step 2: Selection of the process analytical toolboxes
Step 3: Define the design space and design the necessary experiments
Step 4: Multivariate data analysis of the data
Step 5: Define the control system and integrate system into production

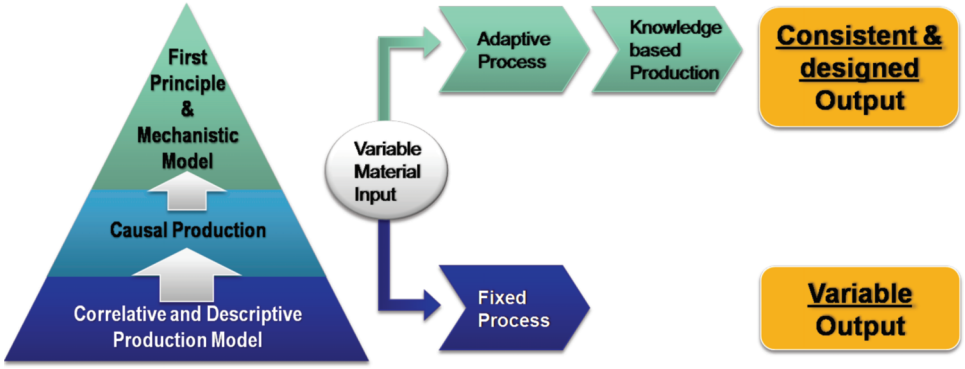


Figure 1.10 Road map for process understanding.

1.3.2 Sampling

In homogeneous systems any sample which is taken will be representative of the whole system. In heterogeneous systems it is difficult to find a way to extract a sample (= fragment) which represents the average of the material. In practice, no sample will be strictly identical to the material as it is a matter of scale [27].

There are four general sampling strategies that are used for process analysis[1)]:

- **Withdrawal**: A portion of the process stream is manually withdrawn and transferred into a suitable container for transport to the analyzer.
- **Extractive sampling**: A portion of the process stream is automatically taken to the analyzer. This may take place either on a continuous basis or at frequent intervals.
- ***In situ* probing**: A probe is inserted into the process stream or vessel and brought into contact with the sample.
- **Non-invasive testing**: Either a "window" into the process stream or another mode of non-contact measurement is used in order to account for interaction of the analysis system with the process material

All four approaches have certain advantages and drawbacks and there is hardly ever a clearly right or wrong approach to sampling.

In *process analytics*, one can distinguish between off-line, at-line, on-line and in-line measurement methods [6]. In the case of *off-line* measurements, samples are withdrawn from the process and analyzed in a laboratory environment which is spatially clearly separated from the industrial equipment. Thus off-line analysis always exerts significant lag times between recognizing and counteracting irregularities. With *at-line* measurements, the sample is withdrawn from the process flow

1) D. Littlejohn, personal communication.

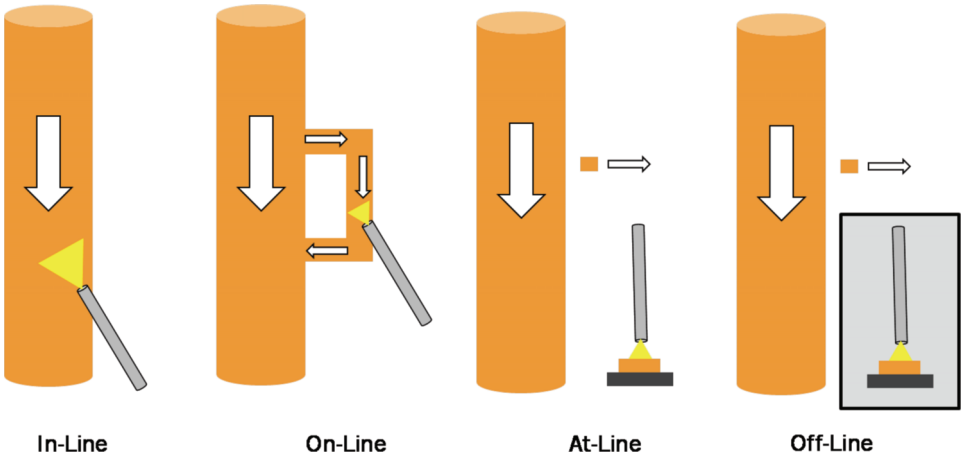


Figure 1.11 Sampling methods.

and analyzed with analytical equipment that is located in the immediate environment of the industrial equipment. Hence, the reaction time for countermeasures is already significantly reduced. Due to the industrial proximity it is often observed that at-line analytical equipment is more robust and insensitive towards process environment but less sensitive or precise than laboratory-only devices. In the case of *on-line* measurements, samples are not completely removed from the process flow but temporarily separated, for example, via a by-pass system which transports the sample directly through the on-line measurement device where the sample is analyzed in immediate proximity to the industrial machining and is afterwards reunited with the process stream. When *in-line* devices are used, the sensor is directly immersed into the process flow and remains in direct contact with the unmodified material flow. Figure 1.11 illustrates various sampling modes realized with in-line, on-line, at-line and off-line sampling. Typical probes for interaction of electromagnetic irradiation with samples are shown schematically in Figure 1.12 for various spectroscopic techniques (transmission, diffuse reflectance, transflection and attenuated total reflectance spectroscopy).

In the ideal case of process control, 100% of the processed elements or products are covered by analytical methods at any time and complete knowledge about the quality of the manufactured goods is obtained throughout the whole process. However, in most cases (when only off-line or at-line devices are used) this is not possible and then *accepted sampling plans* are required which define which and how many samples are inspected at certain intervals [6].

Sampling is always an issue no matter which measurement approach is used, but the nature of the challenge also varies with the approach. For instance, since with on-line analysis a sample may be spatially separated from the main process stream by means of a bypass system, on-line analysis has the advantage over *in situ* or in-line analysis in that the sample can be pre-conditioned (filtrated, extracted, constant

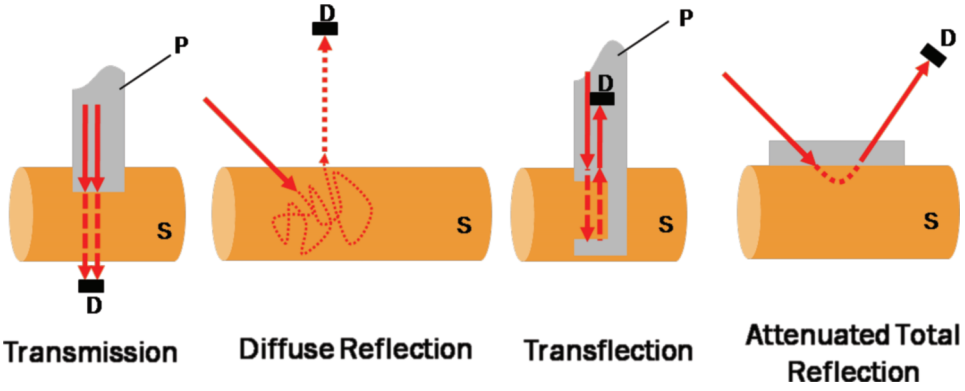


Figure 1.12 Schematic representation of typical spectroscopic probes (D, detector; P, probe; S, sample).

temperature etc.) prior to the analytical procedure. The main concern of sampling is to get access to a representative sample. Besides being representative of the process stream, some additional features need to be considered when selecting a certain sampling approach. Sampling systems must ensure that the sample is[1]:

- obtained and delivered safely,
- conditioned and presented reliably to the analyzer,
- compatible with the analyzer regarding the measurement conditions, such as temperature or pressure,
- obtained in a cost effective way, and
- representative also of the process stage, that is, the lag time from the process to the analyzer must be within an acceptable range

It is generally believed that about 75% of analyzer reliability problems are associated with the sampling system.

Sampling of solids presents the most significant problems. It is clear that finding the key component at low concentrations of a targeted analyte within a complex particulate system with high precision is a real challenge. As a rule of thumb, around 3000 to 10 000 particles are needed to obtain representative values. This can easily be realized when only small particles are present in the medium; however, it is almost impossible to calibrate a system with large particles. In this case, on-line calibration may be the best way to receive representative information over time.

Liquids are considerably easier to sample, unless there are two phases present or the liquids carry high levels of solid compounds.

Gases in general present the least problems, but can still be tricky where there are components close to their dew point. The NESSI consortium has focused on using modular sample system components in building block form that can be linked together to make complete conditioning systems which include pressure regulators, valves, pressure gauges and filters [28].

1.3.3 Process Validation

1.3.3.1 Role of Design of Experiments (DoE)

In January 2011 a new "Guidance for Industry: Process Validation: General Principles and Practices" was introduced by the FDA and will be outlined here [29]. This guidance describes process validation activities in three stages through the whole lifecycle of the product and process, (i) process design, (ii) process qualification, and (iii) continued process verification. These are the basic principles for smart or intelligent manufacturing (see Figure 1.13).

The process should be understood from first principles and from a scientific and engineering point of view. In most cases, the sources of variation during production may already be known by experience; however, only rarely are they really understood or quantified. Thus an important objective of process validation is to attribute all variations in the process and raw materials directly to the product variability. The perfect way to relate product variability to process and raw material changes is to use a DoE strategy [30]. However, it is sometimes difficult to select the appropriate parameters and parameter settings for the design. A parameter is a measurable value which can describe the characteristics of a system, for example, temperature, pressure, and so on. Very often, these parameters may be the factors (= independent variables) which predominantly influence the process and product quality (critical process parameter or factor (CPP)). DoE allows one to identify the relevant factors and to quantify their relative importance. It is logical that the factors which are of importance should be controlled. However, it is sometimes a difficult task to find the correct CPP. Multivariate data analysis (e.g., PCA) of historical data helps to select

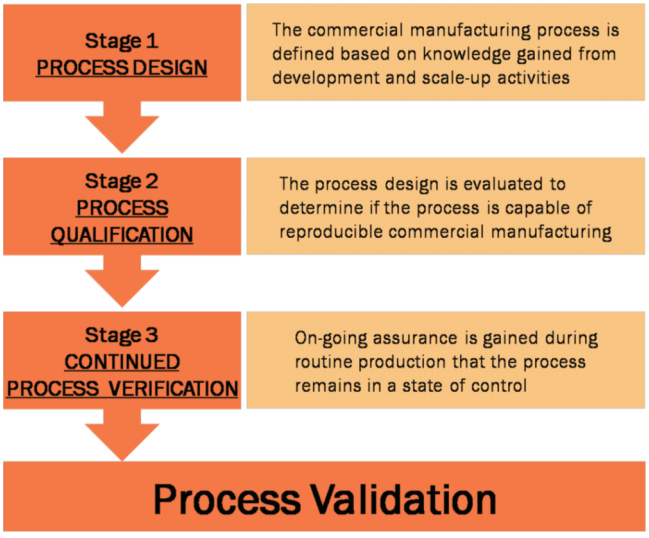


Figure 1.13 Basic principles of smart manufacturing as defined by the FDA [29].

the most important orthogonal parameters [6]. Orthogonality is an important prerequisite in parameter selection for DoE since it is desirable to vary CPPs independently of each other in order to influence the response properties of the product.

Outliers are usually a much better source of information than historical data based on "standard" production, as standard production data only show the variation within the regular production and, therefore, no systematic and significant variation of the factors can be deduced. It is also important to emphasize that a reliable design of the experiments should always include the possibility to evaluate the interaction terms between the factors. When the interaction is more important than the main factors this usually indicates that parameters are used which are not factors and, hence, the DoE has to be modified.

1.3.3.2 Role of Failure Mode and Effects Analysis (FMEA)

Another tool to extract the most important factors is risk assessment such as failure mode and effect analysis (FMEA) or cause and effect matrices [10, 31, 32]. This assists in effectively defining the design space, increases the awareness of the process risks, and yields a better understanding of the relationships between functionality and quality parameters. The critical quality attributes (CQA) are the data that best describe the characteristics that need to be controlled to ensure product quality. Figure 1.14 shows the principal steps involved in FMEA and Figure 1.15 illustrates how FMEA is situated within the production site of a product.

1.3.4 Measurement Technologies (How to Measure)

1.3.4.1 Selection of the Appropriate Technique

One of the key elements for process control is the selection of the best possible technology. Common techniques used in industry measure physical attributes such as conductivity or refractive index. They may be addressed as univariate sensors. Process chromatography can be used to separate the components of complex mixtures, but chromatographic methods are difficult to integrate in *in situ* and

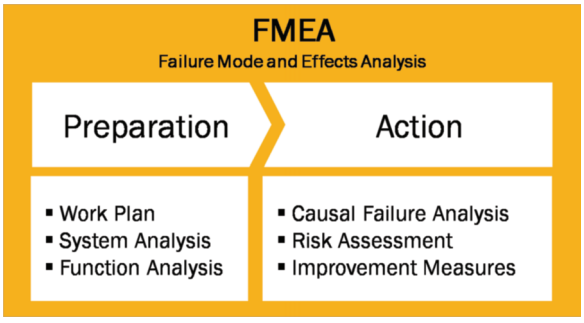


Figure 1.14 Basic approach behind failure modes and effects analysis (FMEA).

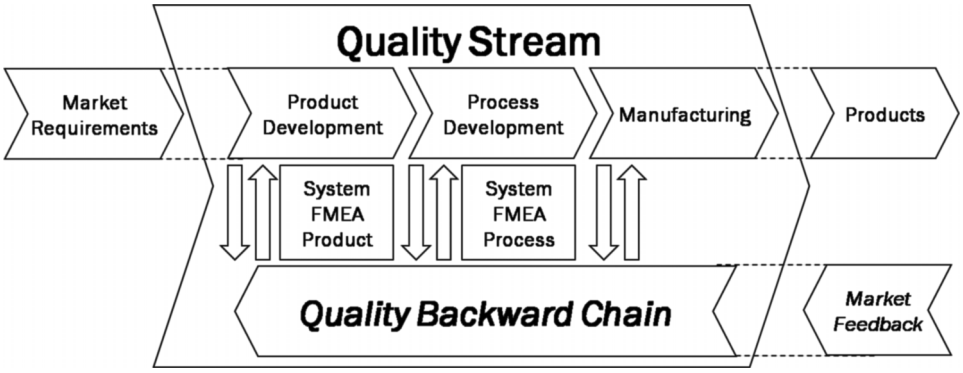
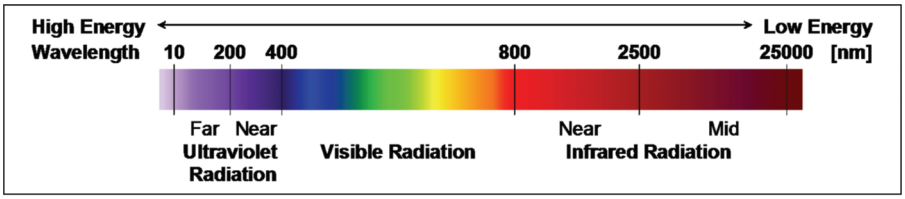


Figure 1.15 Integration of FMEA in the quality stream of a manufacturing facility.

in-line process control set-ups. Unlike optical spectroscopy, new technologies like on-line-NMR or terahertz spectroscopy, as well as mass spectroscopy are not yet standard equipment in in-line process analytics. A detailed overview of the different techniques is given in [6, 33]. Since optical spectroscopy is currently among the most prominent methods used in inline-process analytics, it is discussed in more detail in the following paragraphs.

Optical spectroscopy has developed into a widely used technique in process analytics. Depending on the measurement problem, a broad range of useful wavelength ranges and modes of interaction between electromagnetic radiation and the sample can be used (see Figure 1.16).

Key issues from a practical point of view, besides cost/performance, are the need for high sensitivity and selectivity, as well as the simplicity of application. Although



Fluorescence	UV / Vis - sNIR	NIR / MIR	Raman
Excitation / Absorption: $\lambda \approx$ 200 - 600 nm Fluorescence: $\lambda \approx$ 200 - 900 nm	λ = 200 - 400 nm (UV) λ = 400 - 700 nm (Vis) λ = 700 - 1100 nm (NIR)	MIR: λ = 2500 - 25000 nm $\tilde{\nu}$ = 4000 - 400 cm^{-1} NIR: λ = 1000 - 2500 nm $\tilde{\nu}$ = 10000 - 4000 cm^{-1}	λ_0 = 633 nm $\Leftrightarrow \lambda$ = 633 - 813 nm $\Delta\tilde{\nu}$ = 3500 - 0 cm^{-1}
Excitation /Emission of Electrons	Excitation of Electrons (UV / Vis) and Overtones (NIR)	Excitation of Vibrations, Rotations and Overtones	Excitation of Vibrations Complementary to MIR

Figure 1.16 Selection of appropriate wavelength regions depends on the spectroscopic method used and the intended analytical application.

Table 1.3 Selection of the best possible inline technology in optical spectroscopy [6].

	UV/VIS/s-NIR	NIR	MIR	Fluorescence	Raman
Selectivity	+	+ +	+ + +	+ +	+ + +
Sensitivity	+ + +	+(+)	+ + +	+ + + (+)	+(+ +)
Sampling	+ + +	+ + +	+	+ +	+ + +
Working in aqueous media	+ + +	+	+	+ +	+ + +
Applicability	+ + +	+ +	+	+	+
Process analytical tool	+ + +	+ + +	+	+	+ + +
Light guide	+ + +	+ + +	(+)	+ + +	+ + +
Signal	Absorption	Absorption	Absorption	Emission	Scattering
Sampling on-line/in-line	s, l, g	s, l	s, l, g	s, l (g)	s, l, (g)
Techniques	Transmission Reflectance ATR	Transmission Reflectance ATR	ATR (Transmission)	Reflectance Transmission	Reflectance
Relative costs	1	3–5	6–10	4–6	8–12

the basic layout of spectroscopic tools is always very similar (light sensors–sample contact area–detector), the various optical spectroscopic techniques are based on numerous different measurement principles. Ultraviolet- and visible (UV/Vis) spectroscopy is a highly sensitive technique for electronic transitions while mid-infrared (MIR) spectroscopy is specific for vibrational transitions. Since energy transitions between vibrational states of a molecule are highly substance-specific, peaks measured in the MIR region can be directly attributed selectively to fundamental moieties in a molecule. Near-infrared (NIR) spectroscopy is less sensitive due to lower yields of the higher order vibrational transition probabilities. However, although not easily directly interpretable, the major advantage with NIR is that, even at higher concentrations, no sample preparation (e.g., dilution) is needed. It is important to emphasize that both NIR and MIR spectroscopy are highly sensitive to water absorption. Table 1.3 shows a qualitative comparison of the advantages and disadvantages of the different optical spectroscopic tools.

1.3.4.2 **Working in Aqueous Systems**

Figure 1.17 shows the spectra of water in the NIR and MIR wavelength ranges. Due to the different absorption cross sections of the fundamental vibrations (MIR), the combination bands, first second and third overtones in the NIR, different path lengths must be used. The measured MIR spectrum is measured with a diamond ATR system with a mean path length around 5 μm.

The strong water absorption limits a broad application of these techniques in aqueous systems, for example, the study of fermentation processes. Raman spectroscopy may be advantageous over NIR and MIR spectroscopy in aqueous systems. In recent years, Raman spectroscopy has developed into a highly sensitive and versatile technique and, therefore, has proven a very suitable method in biotech-

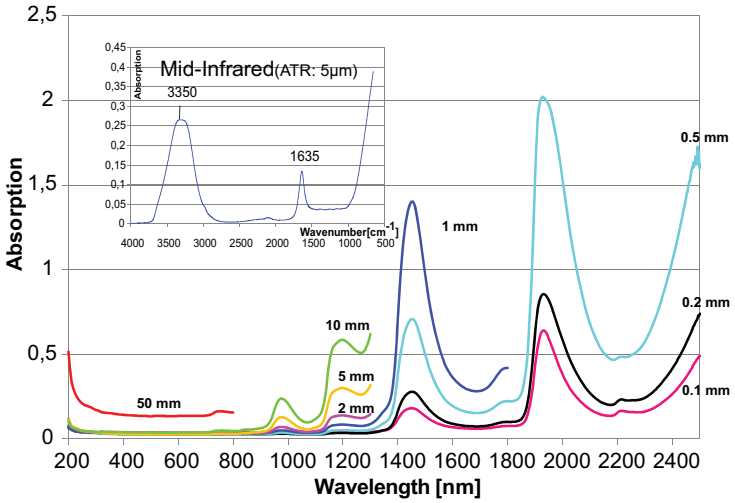


Figure 1.17 Absorption spectra of water in the NIR region with different path lengths and MIR (insert).

nology. For special applications fluorescence spectroscopy is certainly one of the most sensitive techniques in spectroscopic analysis.

1.3.4.3 Trace Analysis

Trace analysis is still a challenge in process analytics. Optical spectroscopy can cover a broad range of sensitivities and selectivities, as described before. One major advantage of NIR is that the absorption cross sections are generally low. Thus the technique can be used even at high concentrations. The typical detection limit for low concentration mixture components lies at around 1%; due to the high absorption coefficient for water as a trace component it is in the range down to 0.1% (or even 0.01%, depending on the system). In contrast, with MIR spectroscopy concentrations as low as 0.01% are easy to measure and standard detection limits can be even as low as 0.001%. Although Raman absorption cross sections lie typically around 10 orders of magnitude lower than in FTIR, due to recent development of extraordinarily sensitive detection systems Raman spectroscopy may approach the performance of FTIR spectrometers in the near future. UV/Vis and fluorescence spectroscopy are very sensitive techniques (in ppm, ppb and even lower), but lack selectivity.

As can be seen, the work horses in PAT are in many cases sufficiently sensitive. However, especially for applications in biotechnology when one is working in an aqueous environment at typically rather low metabolic concentrations, only chromatography in combination with mass spectroscopy may be a reasonable option [6, 33].

1.3.4.4 Qualification of a Spectrometer

Generally the quality of a spectroscopic inline control system can be described in terms of its spectral range, spatial resolution, non-linearity, S/N ratio (stray light),

diffraction efficiency and stability. The parameters needed to characterize the systems are, for example:

- Spectral resolution
- Spectral linearity
- Absolute efficiency of the optics (throughput) and diffraction efficiency of the grating,
- Straylight (S/N), ghost line and ghost image properties.
- Wavelength stability

Spectral axis calibration is done with spectrally well known light sources for example, neon lamps, lasers, fluorescent systems. The background CCD signal (dark current) must be compensated for and – if possible – be minimized by cooling. The detector response to light varies from pixel to pixel and is also strongly wavelength-dependent. Moreover, the energy throughput of lenses and other optical elements also depends on the wavelength. These variations can be calibrated by measuring a white reference surface, storing this image, and then calculating the ratio between a measured sample image and this white image (after dark current subtractions). Light source color temperature drift and lighting spatial non-uniformity are also compensated for, as long as the texture of the reference and the target is similar in terms of specular and diffuse reflectance.

The spectral range defines the wavelength regions covered by the spectrometer. The spectral resolution is related to the monochromator system used and defines the power to resolve the narrowest spectral features in the electromagnetic spectrum. The bandwidth is defined as the full width at half maximum of the spectral line. It is important to notice that the optical resolution is different to the (digital) pixel resolution of, for example, a diode array spectrometer. The pixel resolution describes the number of digital points which are required to represent a peak in the spectrum. Usually the pixel resolution should be about 2 to 3 times higher than the optical resolution. The signal to noise ratio is the ratio of the radiance measured to the noise created by the instrument electronics and the detector.

For on-line process analysis some additional features like the frequency of maintenance and the frequency of recalibration are important and define, among other features, the cost of ownership. Details of the calibration procedures are defined in ASTM standards. The location of the analyzer must be compatible with the safety ratings of the end user area.

1.3.5
Data Analysis and Calibration (How to Process Data and How to Calibrate)

1.3.5.1 Introduction

The basic idea of multivariate data analysis is to extract useful information from data and to transfer this information into knowledge. Figure 1.18 visualizes the methodology of multivariate data analysis to extract useful information from multidimensional data sets.

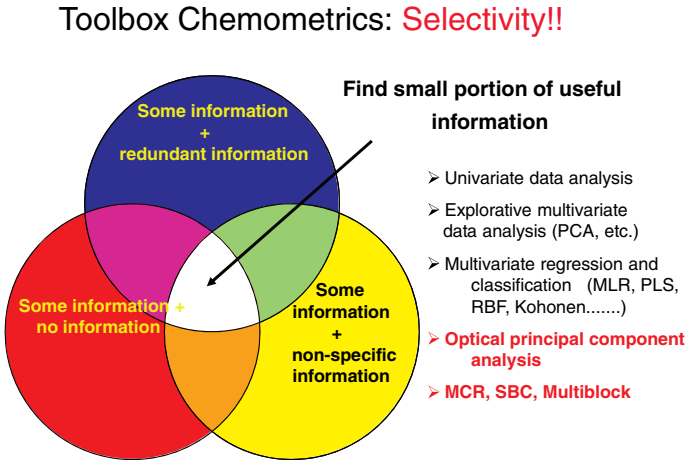


Figure 1.18 Extraction of information from a large data set.

In common data there is high redundancy of the information, overlapping with no-information (white noise) and information which is of no use for the specific problem. Besides univariate data analysis, the chemometric toolbox includes explorative data analysis like principal component analysis (PCA); multivariate regression analysis like partial least square analysis (PLS); and nonlinear approaches like neural networks. It is important to emphasize that the first step for a proper calibration and modeling is always the correct optical set-up of the spectrometer device and the appropriate definition of the measurement procedure. As will be shown later, complex systems are preferably analyzed and described by using multiple spectroscopic methods (multi-modal spectroscopy) which may be addressed as "optical principle component" analysis. Hybrid models like multivariate curve resolution (see below) or science-based calibration (SBC) allow the introduction of knowledge into the modeling.

A standard procedure to extract information and transform this information into knowledge may be:

- Standardization and calibration of the instrument
- Spectral data pretreatment
- Data cleaning
- Principal component analysis
- Regression analysis
- Evaluation and figures of merit

The procedure for standardization and calibration of the instrument was described in the previous section.

1.3.5.2 Spectral Data Pretreatments and Data Cleaning

As common in spectroscopy, the measured dark spectra, reference spectra and sample spectra are used to calculate the corrected sample spectra. Data transformation may

then involve the conversion of the raw data into, for example, absorbance. The measured diffuse reflectance spectra can be transformed to absorbance or Kubelka-Munk units in order to linearize the correlation to chemical constituent concentrations.

Changes in sample surface, sample orientation, particle size distributions, compaction of loose samples like powders and external changes in the illumination or the detector response (for instance by temperature drift) may result in unwanted spectral signals, which are added or subtracted throughout the whole spectral range. To reduce these additive effects a first and second derivative can be carried out. If the spectra are very noisy they have to be smoothed before calculating the derivatives. A detailed overview of standard pretreatment procedures and their effects on the optical spectra is given in [34].

Spectra normalization, either to length one or area one, can be a choice if the interesting information is more related to the shape of the spectral features than to changes in absorbance intensity due to concentration variations of a constituent. In such cases when classification (qualitative information) is aimed at, normalization is a very helpful pretreatment method since the spectra will become independent of their global intensity.

To correct for particle size or other scattering effects a multiplicative signal correction (MSC) can be applied [35, 36]. Several methods have been described in the scientific literature, ranging from simple MSC to more sophisticated methodologies such as extended MSC [37] and stepwise multiplicative scatter correction (PMSC) [38]. Alternatively, the standard normal variate (SNV) correction for scatter effects can be used. SNV is a simpler but purely mathematical-based procedure to correct for scatter.

In order to correct for baseline curvature or other nonlinear effects across the NIR spectral range a de-trending algorithm can be applied subsequently after an SNV transformation. Barnes *et al.* [39] have shown that MSC and SNV give more or less the same results.

1.3.5.3 **Chemometrics**

Chemometrics offers the possibility to extract relevant information from multiple wavelengths and methods instead of using single-wavelength channels only. Additionally, chemometrics reduces this relevant information into one or a few quality defining parameters (underlying entities) by applying either multivariate classification or regression models to the data.

There has been constant development in chemometrics and a number of good reviews and useful tutorials have been published [34, 40–42]. The advent of modern computer systems in the past decades has boosted widespread use of chemometric software packages, and has also had a very positive effect on the broader distribution of mathematics-intensive spectroscopic on-line methods.

Principal component analysis is a chemometric method that decomposes a two-dimensional data Table X into a bilinear model of latent variables, the so-called principal components, according to the following expression:

$$X = TP^T + E \tag{1.1}$$

where T is the scores matrix and P^T the transposed loadings matrix. The matrix E is the residual matrix and accounts for the experimental error (noise), which is not part of the model. The principal components are calculated so that they explain as much variance of the data as possible. The first principal component captures most of the variance in the data set. This information is then removed from the data and the next principal component is calculated, which again captures the major part of the remaining variance; this procedure is continued until a pre-defined stopping criterion is met, which is based on falling below a lower limit of variance explained by an addition of another principal component. All principal components are linearly independent (orthogonal); that means there is no correlation among them and they can, therefore, serve as a new coordinate system with reduced dimensions.

A so-called loading plot shows the relative importance of the individual variables (here: absorbance at different wavelengths). It can be used to assign the spectral classification to molecular structures of the chemical components. The objects can also be represented by their scores in the new principal components space. This allows clustering and structuring the samples quantitatively.

The fact that the principal components have no correlation among each other, as they are calculated to be orthogonal, results in negative scores and loadings. This makes it often difficult to interpret the underlying chemistry. To overcome this deficiency, MCR can be applied instead, where non-negativity is one of the basic prerequisites for calculation. Such MCR methods have been introduced to image analysis only recently, with growing attention and success. More details can be found in [9, 40].

1.3.5.4 **Regression Analysis**

Regression The target of PCA is more explorative but it is well possible to build regression models with the PCA scores regressed on target values. This is called principal component regression (PCR). However, as in traditional spectroscopy, the most commonly used algorithm for multivariate regression is partial least squares (sometimes also called projection to latent structures). The PLS algorithm builds an inverse calibration model for the spectra X and the target value Y according to the following regression equation:

$$Y = XB \tag{1.2}$$

The matrix X contains the spectra and Y holds the corresponding target values, which are the properties to be predicted. Y can be a matrix, but especially in process control it often has only one y-variable. PLS uses latent variables, similar to the principal components in PCA, to build the calibration model. The latent variables are calculated so that they explain as much as possible of the covariance between X and Y.

The model size (number of latent variables) is determined by the internal validation data set and is checked for correctness with an external data set. The figures of merit are given as bias and root mean square error of prediction as a measure of accuracy and precision. They are calculated separately for the different data sets according to the following formulae:

$$bias = \sum_i (y_i - \hat{y}_i) \tag{1.3}$$

$$RMSEP = \sqrt{\frac{\sum_i (y_i - \hat{y}_i)^2}{n}} \tag{1.4}$$

where y_i is the reference concentration for the ith sample (given by the reference method), $\hat{y}_i$ is the predicted concentration by the calibration model and n the number of samples. When the model has been validated, it can be used to predict y values (e.g., concentration) for measured spectra.

Evaluation of the Calibration The reliability of a method includes accuracy and precision. "*Accuracy*" in testing means "closeness to the true value". Especially in biotechnology, this is hard to define because usually the relevant constituents cannot be prepared in a pure state and their spectral characteristics depend strongly on the interfering matrix material. Within a laboratory, accuracy can be established by repeated analysis. Between laboratories, accuracy can be assessed by using the mean results of collaborative studies (ring tests) among all of the laboratories belonging to the same organization. "*Precision*" in any testing means obtaining the same result every time the measurement is repeated. It includes repeatability and reproducibility. "*Reproducibility*" includes all features of the test, including sub-sampling, sample preparation and presentation to the instrument, and testing by all of the operators that are likely to be involved in the testing. It is determined by repeated analysis of the same sample, including all of the steps involved in the analytical procedure and all of the operators likely to be involved in future testing. "*Repeatability*" includes all features of the test except sub-sampling and sample preparation. It is determined by performing duplicate or replicate tests on the same sample, after sub-sampling and sample preparation, and is a test of the actual method on a single sample after sample preparation. It is important to emphasize that PLS can be misleading if it is not used with care [43, 44]. To select the correct validation technique is the key for causality (see Section 3.1).

1.3.6
Process Control (How to Control a Process)

As described in the "Guidance for Industry: Process Validation: General Principles and Practices" and other papers, process analysis provides quantitative and qualitative information about a chemical process in real time, using on-line and in-line analyzers [29, 45]:

"The information given by these systems is used to control the process. The control strategy is defined as the input material controls, process controls and monitors, and finished product tests, as appropriate, that are proposed and justified in order to ensure product quality. The control strategy will ensure the product is manufactured within the Design Space to meet all Critical Quality

Attributes and other business-driven quality attributes (e.g., that affect cost or manufacturability)".

Process control is important for economic, safety and environmental reasons. Improved process control allows more efficient use of feedstock and energy, giving better product quality and ensuring consistency of quality. It also enables improved treatment of waste products and effluent to meet continually more stringent environmental legislation.

The key to good process control lies in the ability to measure fluctuations in the system behavior (e.g., changes in feedstock composition or temperature build-up). This information is then used to compensate for these changes and to optimize process parameters. How representative measurements are of a system, how long the interpretation of the data takes and how quickly this information can be acted on, are important factors of process control.

In this section, the strategy of a modern manufacturing using feed-back and feed-forward control is described. Figure 1.19 visualizes the strategy for process control and how to manage variability.

Process control summarizes all measures to keep quality within certain limits. *On-line statistical process control* involves actions to monitor deviations from a desired state while the process is actually running and manufacturing takes place. Hence, in order to be able to react promptly in response to observed deviations, real-time monitoring techniques are advantageously employed. Even when a process is well designed, statistical process control measures are always useful, since they allow one to detect and act upon unforeseen effects of immediate or abrupt changes in the process

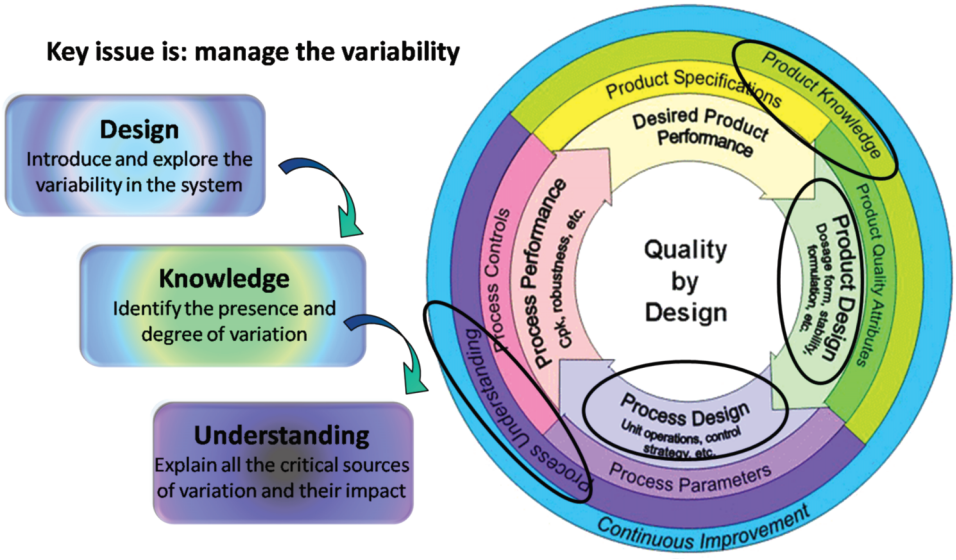


Figure 1.19 Managing variability (modified after FDA).

conditions, and to correct the process for statistical variations that occur, for instance, in the raw material, which is always likely to happen. Moreover, processes when first implemented are usually not yet optimized with regard to the highest possible level of quality they might produce; on-line statistical process control may well assist in such optimization and continuous improvement. Regarding the information that is used for statistical process control, laboratory data or process data may be used. *Laboratory data* typically comprise physical or chemical tests performed on the incoming raw material to control the input quality, or technological tests for the application behavior of the final products to control the output quality. Especially in the early phases of industrial manufacturing, this was the main mode of controlling the overall performance of a process, the major indicator of process quality being the amount of waste production or sorted-out parts (Figure 1.2). Regarding process-related data two different types of *process data* are available: the first includes machine data, which are accessible by recording parameter settings of the machine equipment, or general, unspecific process data, like temperature, pH or conductivity, which may be recorded by various sensors throughout the whole course of the production. The second type includes quality influencing, material specific data which are directly related to and measured on the produced goods by means of process analytical methods which are applied at numerous intermediate product stages. Traditional laboratory data alone, especially when only applied as end-of-pipeline tests of product performance, only allow *feed-back control* loops, that is, after having detected that a fraction of the final

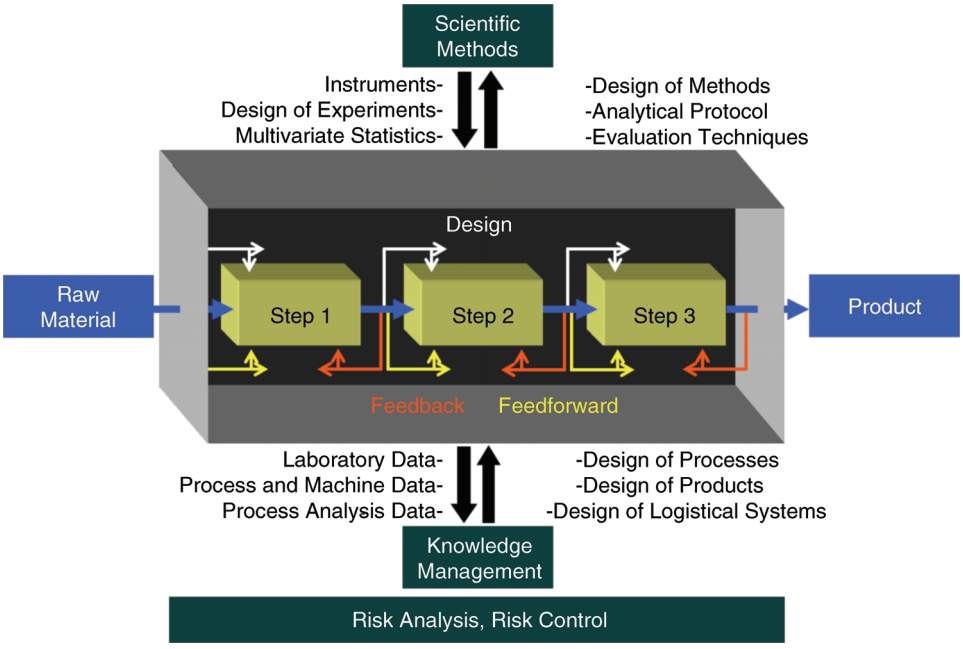


Figure 1.20 Process control by feed-back and feed-forward control requires a holistic view of the process and the trans-disciplinary interplay of numerous techniques and methods [46].

product is unusable and must either be downgraded or even discarded, the process is modified until subsequent batches may again be within specifications. In contrast, process data (in combination with a causal understanding of the overall process) may more intelligently be used in so-called *feed-forward control* loops which allow anticipation of product properties before they have actually manifested. In this way, correcting measures may be undertaken in advance in the case of expected negative deviations and inferior or waste production may be totally avoided. Figure 1.20 (adapted from [46]) summarizes these concepts schematically.

1.4
Specific Problems Encountered in Industrial Process Analytics

In this section, selected process analytical problems that are of crucial importance to many applications throughout various branches of industry are addressed. Since they are often encountered in an industrial environment, this section is dedicated to principle problem solutions that deal with moisture determination, the process analytics of solids and surfaces, strongly scattering systems, and the spatially resolved spectroscopy of samples using spectral imaging.

1.4.1
Moisture Measurements (NIR, MW)

Moisture is an important quantity which influences processability and shelf-life. Moreover, delivering a product of defined moisture content not only saves energy but also increases profit. Off-line moisture measurements are time-consuming and cannot be used for a feed-back or a feed-forward control in manufacturing. Therefore, there is a strong demand in industry for on-line and in-line control of water in a substrate [47].

1.4.1.1 NIR Spectroscopy

As described in the previous chapter, MIR and NIR spectroscopy show strong absorption for water and, therefore, can be used for in-line analysis of moisture content. NIR spectroscopy is based on measurements of light absorbed by the sample when it is exposed to electromagnetic radiation in the range from 780 nm (12 820 cm^{-1}) (short-NIR, s-NIR) to 2500 nm (4000 cm^{-1}) (NIR). As described in the previous chapter, water absorption in the NIR occurs mainly at wavelengths around 1445 and 1900 nm (Figure 1.21).

There are numerous investigations which deal with the determination of surface water and intrinsic water (bulk water). To separate these two kinds of water spectroscopically, it is generally postulated that surface water predominantly absorbs around 1900–1906 nm and intrinsic water, respectively bound water, at 1936 nm. However, bands in the NIR are broad and, in most cases, spectrometers were used which were limited in their optical resolution. Thus care must be taken to interpret spectra obtained from measurements on real life components.

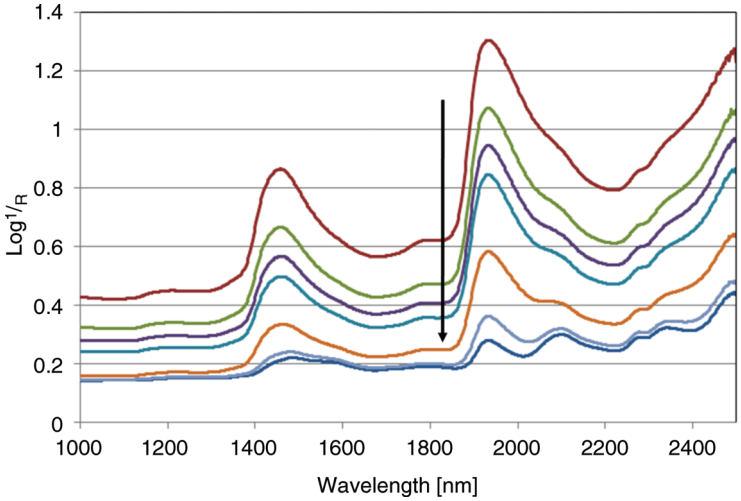


Figure 1.21 NIR spectra showing the typical absorbance pattern of water with maxima at 1450 and 1950 nm. The spectra show the evaporation of water from cellulose-based filter paper during drying. Measurements were performed with a Lambda 1050 NIR spectrometer (Perkin Elmer) in diffuse reflectance using an integrating sphere (150 mm).

Furthermore, as described in the next chapters, the cross sections for absorption, and also for scattering, are usually in a range of low penetration depths of photons. At strong absorptions in the NIR (e.g., combination vibration), penetration and, hence, information depth may, therefore, be only a few hundred microns or even less; in the third overtone of water (s-NIR), penetration into the substrate may go up to several millimeters. Therefore, the measurement of moisture using NIR combination bands is usually restricted to the determination of surface water. The typically used reference measurement techniques for calibration of the spectroscopic signal, such as Karl Fischer titration or gravimetric methods ("loss on drying"), however, determine the overall bulk moisture content, which does not necessarily correlate to the surface moisture that is measured by NIR spectroscopy. Hence, calibration of NIR spectra may be erroneous. Moreover, NIR spectra often also contain spectral contributions from other components which may overlap with water peaks. To make things even more complicated, signals may also be perturbed by scattering due to particles of different (and unknown) size distribution, resulting in nonlinear mean free path length variations of the photons. In multivariate calibrations, SNV, MSC or EMSC have been successfully used to eliminate baseline offsets present in the raw spectra and can compensate for differences in thickness and light scattering of the analyzed samples.

1.4.1.2 **Microwave Resonance Technique (MWR)**

In the case of microwave resonance (MWR) measurements the specific absorption of water in the microwave wavelength range of 2–3 GHz is used. Sensors are designed from cavity resonators or stray field resonators. The penetration depth of the microwaves is in the several-cm range and, therefore, bulk moisture measurements

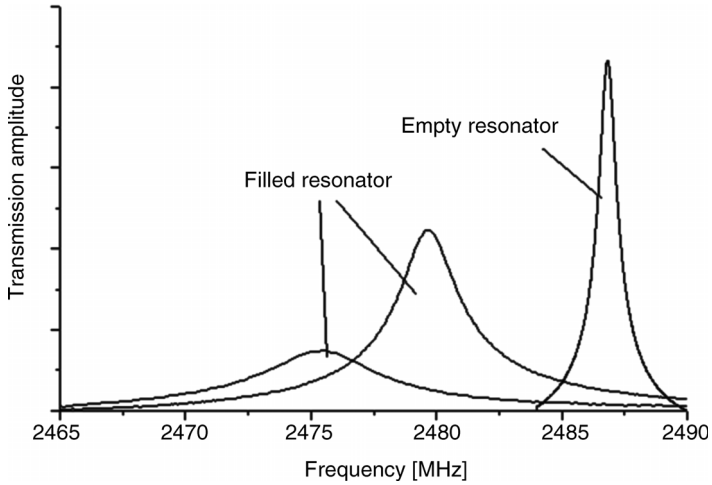


Figure 1.22 Microwave spectra of an empty and filled resonator.

can easily be correlated. Figure 1.22 shows an example of the microwave spectrum of an empty resonator and a resonator filled with a substrate.

Usually, the spectrum of the empty resonator is stable and can be used as an "absolute" reference which is specific for a given system and system set-up under certain temperature-controlled conditions. When a moisture-containing substrate is measured (surface and bulk), the spectrum of the empty resonator changes in two ways: First, the resonance spectrum shifts towards lower frequencies due to the dielectric losses and, secondly the spectral bandwidth increases in response to the density, respectively, the mass of the substrate. This information can be used to compensate for fluctuations of the measured path lengths due to particle size variations or other density changes (like, for instance, compaction) during on-line measurements. Hence, besides being a very precise and reproducible measurement method for moisture content, MWR also allows mass or density determination of the bulk material which is corrected for the moisture content (dry mass determination). Being a very rapid measurement method, the MWR technique allows up to 500 single measurements per second of powders, granules, fibers and even solids. This allows easy averaging of the spectral information. Unlike other dielectric techniques, such as capacitive techniques, conductivity measurements or microwave transmission measurements, unperturbed information is obtained, even in cases where substrates are used that contain high amounts of ionic material. Numerous applications can be found in the literature for food and feed control [48, 49]. Figure 1.23 shows examples for the set-up of on-line measurements in the food industry.

1.4.2
Process Analytics of Solids and Surfaces: Specular and Diffuse Reflectance

In real-life samples of solids or surfaces, reflectance spectra are composed of contributions from specular and diffuse reflected light. Pharmaceutical tablets show

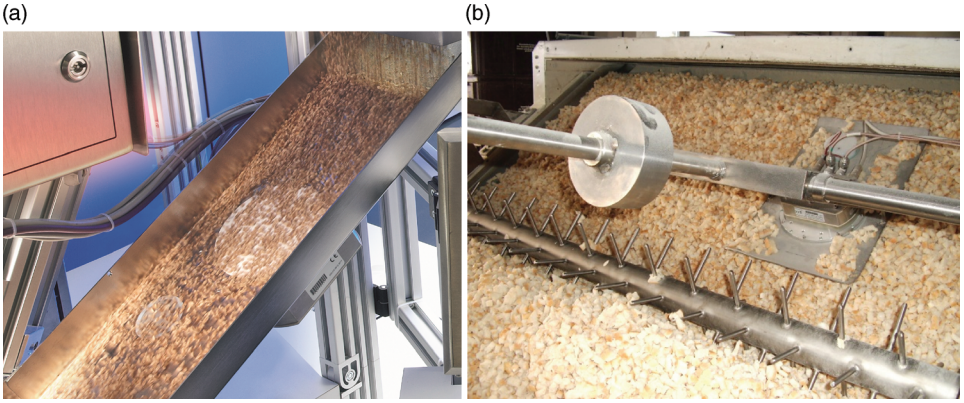


Figure 1.23 On-line control of wheat grains by a microwave stray field sensor (a) and a slide sensor measuring moisture of bread crumbs (b), (courtesy of Sartorius).

primarily diffuse reflectance but, due to different degrees of compaction, specular surface reflectance is also observed. Decisive changes in the scattering coefficient occur during compaction and relaxation and have a great impact on the measured signal. Metal surfaces, on the other hand, exhibit mainly specular reflectance and interference; however, they may also show some diffuse reflectance due to defects within the layer, surface roughness, or contaminants on the surface. In a more complex system like wood chips, on-line control allows correlation of the spectral signature not only to a single target value like lignin, but to a complex quality definition like functionality.

The Fresnel equations are the basis for the calculation and interpretation of the portion of light which is specular reflected from optically smooth surfaces and consists of wavelengths that are comparable to those of the incident light. Diffuse scattering originates from surface irregularities that are of the same order of magnitude (or slightly smaller) as the wavelength of the irradiating light source, as shown schematically in Figure 1.24. Diffuse reflected light is described by the

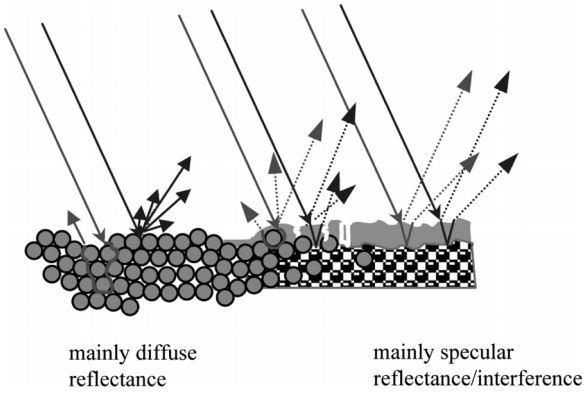


Figure 1.24 Specular and diffuse reflectance of a real-life sample surface [50].

Kubelka Munk function [50]. In this case, diffuse reflectance is influenced by both the absorption and scattering coefficients, which are usually denoted as k and s. In an ideal case, the absorption coefficient k and the scattering coefficient s can be separated by the measurement of a sample with defined thickness in diffuse reflectance on a highly scattering white or black background, or by means of diffuse transmission and reflectance spectroscopy [50].

In real-life samples, specular and diffuse reflectance contribute simultaneously to the overall spectrum, which is then a mixture of both effects. A detailed mathematical description of the resulting solutions of the Fresnel and Kubelka Munk function is given in [51].

The ideal experimental set-up would be to measure the reflectance by means of an integrating sphere. But integrating spheres are not suitable for in-line or on-line control. Moreover, the analytical deconvolution of the spectral information into the contributions of the pure specular and diffuse components is not a simple task. Thus, efforts must be made to focus the measurement on either diffuse reflectance or specular reflectance in order to optimize the response, depending on the required physical or chemical information.

An even stronger discrimination between diffuse and specular reflectance is feasible using polarized light. Polarized light is depolarized by scatter centers and therewith the information on defect sites is emphasized.

A specific target of the PAT initiative of the Food and Drug Administration of the United States of America is to identify and quantify an active pharmaceutical ingredient (API) in a complex formulation. Thus a direct relation between the measured spectrum and the concentration of the API must be established at high precision. The objectives of on-line control are, therefore, a fast and robust noninvasive measurement protocol which is not perturbed by artefacts. Pharmaceutical tablets are made from small particles which can act as ideal scatter centers for diffuse reflectance. Spectra, therefore, simultaneously show (i) wavelength-dependent scattering, and (ii) specific absorption due to their chemical composition. Since the process of tablet formation leads to a smooth surface after compaction, the tablets additionally exhibit specular reflectance which perturbs the diffuse reflectance measurement. For on-line control, the amount of specular reflected light being transmitted to the detector should be minimized. A possible set-up to bring this about is the diffuse illumination of the sample and a detection perpendicular to the illuminated surface. Alternatively, the illumination can be performed at an angle of 45° while the detection of the diffuse reflected light is again done at an angle of 90° to the surface (assignment: 45R0). A detailed description is given in [52].

1.4.3
Working in Multiple Scattering Systems: Separating Scatter from Absorbance

1.4.3.1 Basics in the Measurement of Opaque Systems

In scattering systems, the interaction of light (photons) is complex and includes refraction, specular and diffuse reflectance and/or transmission, as well as absorption and scattering simultaneously. Due to the diffusion of photons, even the spatial

identification and attribution to a defined spatial coordinate in x and y may diminish. Hence, the spectroscopic investigation of samples that contain phase boundaries and, therefore, simultaneously display absorption and scattering effects show several restrictions regarding the experimental procedure (methodological approach) and the substrate:

Methodological influences

- Angle of illumination and detection (specular and diffuse light)
- Wavelength range: for example, UV–VIS–NIR–IR
- Polarization of the light (illumination and detection)
- Illumination and detection area
- Focal planes of illumination and detection

Substrate influences

- Differences in particle and matrix refractive indices
- Particle size
- Particle size distribution
- Volume concentration – compaction
- Scattering and absorption coefficients

Figure 1.25 shows schematically the different effects occurring when photons interact with an opaque substrate.

Particles produce scattered light. The intensity of the scattered light is dependent on the size of the particle and the wavelength of the interacting photons. Smaller particles show higher scatter and light of shorter wavelengths is more strongly scattered than light of longer wavelengths. This results in a difference in mean free path lengths and penetration depths of the photons. In combination with absorption phenomena, the overall spectral response will be significantly different when the same sample is measured in diffuse reflectance or in diffuse transmission. Also, the thickness of the measured sample will have a strong influence on the obtained spectra due to photon loss at the rear side of the sample. These photons cannot be

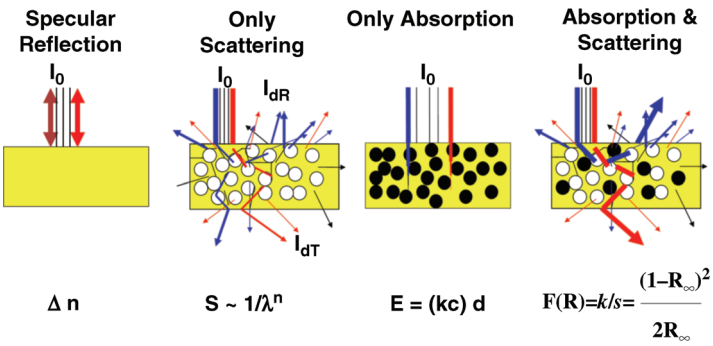


Figure 1.25 Schematic representation of the different possibilities for the interaction of photons with opaque systems.

scattered back again as in the case of infinite sample thickness. Thus, the geometrical set-up of the measurement is an important factor and changes in particle sizes and size distribution must be compensated during calibration.

It is also important to emphasize that scattering also changes the direction of the photons. Theoretical calculations have shown that the travel distance of the photon may even be expressed in millimeters and more. Therefore, in highly scattering systems, information from one spatial region may be influenced by information from another region, resulting in a mixing of the spatially separated spectral information. As a result, there is a trade-off between spatial resolution and chemical (quantitative) information [6].

1.4.3.2 **Separation of Scatter from Absorption**

In many chemical, pharmaceutical and biotechnological processes not only the chemical changes are of importance but also the morphological variation of the particulates. When spectroscopic in-line control is applied to such complex systems very often the scatter in the spectra is regarded as unwanted and, therefore, eliminated by chemometric methods instead of using it as a supplementary source of information on the morphology of the substrate. One of the most appropriate theories to describe multiple scattering and absorption is the radiative transfer equation (RTE). A summary of RTE is given in [53]. A survey of the different techniques is described in [54].

Using this equation, three separate and independent measurements are necessary to separate scatter from absorption. Kortüm [50] and coworkers have demonstrated extensively the power of diffuse reflectance spectroscopy in quantitative measurements of turbid systems. The simplest approach is to use the Kubelka Munk (KM) function, where $F_{(R\infty)}$ describes the reflectance of an optically infinitely thick sample.

Linearity between the spectral response and concentration is only obtained (i) with a specific optical set-up for diffuse illumination and (ii) when a defined preparation procedure of the samples is used with special consideration for reproducible grinding and dilution of the sample with powders that do not absorb. In practice, the alternative $\log 1/R_\infty$, that is called "absorbance", prevails over $F_{(R\infty)}$ of the KM function in most publications. The KM function is shown in Eq. (1.5). Here K stands for absorption and S for scattering

$$E(R_\infty) = \frac{K}{S} = \frac{(1-R_\infty)^2}{2R_\infty} \tag{1.5}$$

Several models exist to determine the wavelength-dependent scattering and absorption coefficients in diffuse reflectance and diffuse transmission spectroscopy [19]. K and S can be calculated for example, from the simplified solution of the differential equations of the Kubelka Munk function of a light flux into and from the sample. Eqs. (1.6) and (1.7) describe the relation between S and K with the measurements of a sample in diffuse reflectance.

$$S = \frac{2.303}{d} \cdot \frac{R_\infty}{1-R_\infty^2} \cdot \log\left(\frac{R_\infty(1-R_\infty \cdot R_0)}{R_\infty - R_0}\right) \tag{1.6}$$

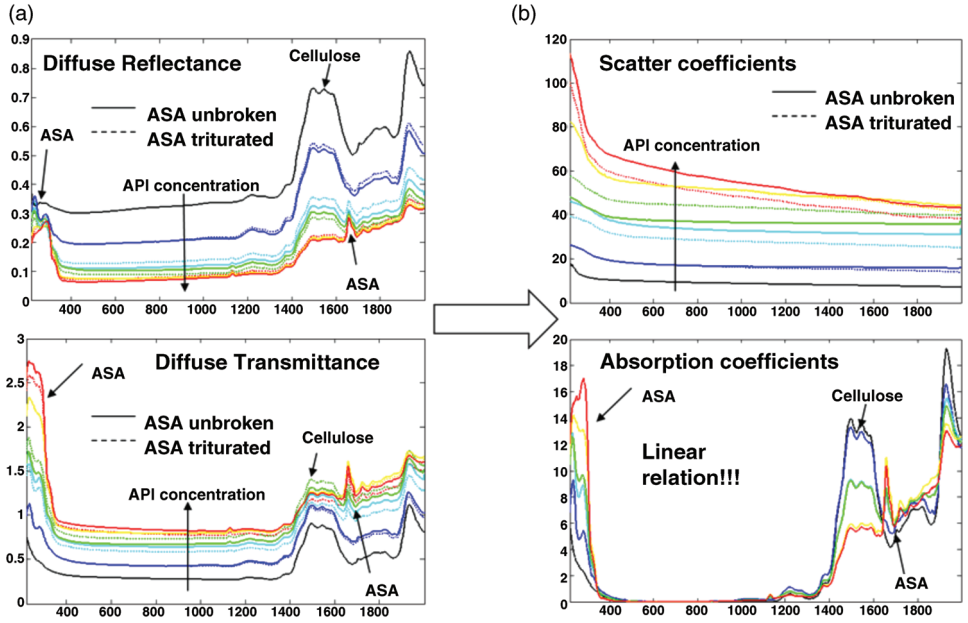


Figure 1.26 Diffuse reflectance and diffuse transmittance spectra of Aspirin in cellulose (a) and calculated wavelength-dependent scatter and "pure" absorption spectra (b) [52].

$$K = \frac{2.303}{2 \cdot d} \cdot \frac{1 - R_\infty}{1 + R_\infty} \cdot \log\left(\frac{R_\infty(1 - R_\infty \cdot R_0)}{R_\infty - R_0}\right) \tag{1.7}$$

Here, R_0 denotes the spectrum measured at a definite sample thickness with a black, strongly absorbing background and R_∞ denotes the measurement of the same sample with an ideal white scatter (= nonabsorbing material for example, barium sulfate) as a background. Other approaches are described by Oelkrug, Dahm and Dahm [55, 56].

Figure 1.26 shows the result of a spectrum where the contributions of scatter and absorption have been separated. For demonstration, an aspirin tablet with cellulose as excipient is measured at different compactions in diffuse reflectance with infinite and definite thickness. For details see [6, 9, 52].

As can be seen clearly, the absorption portion of the spectrum in the longer wavelength region containing chemical information is slightly perturbed by scatter, whereas in the shorter wavelength range, scatter is the dominating information source on the morphology of the sample.

This deconvolution can also be used with advantage in chemical imaging. Figure 1.27 shows the image of an aspirin (ASS) tablet as measured at the specific absorption wavelength of ASS in diffuse transmission and diffuse reflectance. The S and K values were calculated at the specified wavelengths.

The figure shows nicely that the same particle of ASS shows different regions of absorption depending on the geometrical set-up of the measurement and the used

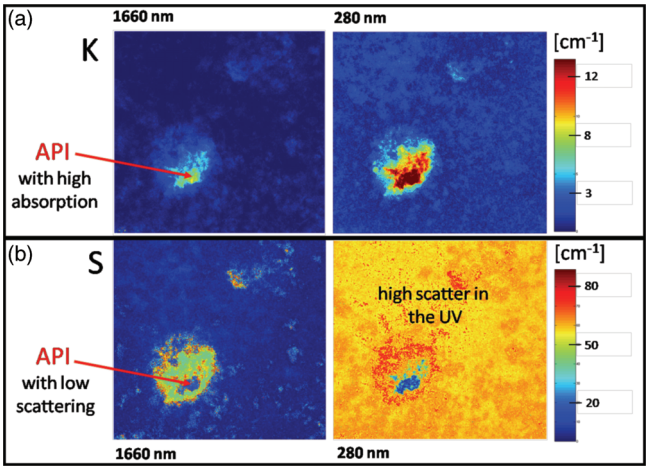


Figure 1.27 Spectral imaging of an aspirin particle in cellulose: (a) "pure" absorption of the aspirin at different wavelengths, (b) "pure" scatter of the particle at different wavelengths (for more details, see [52].

wavelength. UV absorption is significantly stronger than the absorption in the NIR range. This is also true for the scattering, which at lower wavelengths is much stronger than in NIR. It can also be seen that scattering is especially strong at the phase boundaries of the particle.

Figures 1.26 and 1.27 clearly show that the overall spectrum is the superposition of the contributions of absorption and scattering effects. Therefore, the mean free path lengths of a photon into and through a tablet will depend on the number of scattering centers and the scattering characteristics of the particles. The probability of interaction of the photon, and thus the signal intensity, also depends on the portion of photons that is absorbed during their motion through the particulate system. Certainly, the measured intensity also depends on the layer thickness since photons are lost by transmission when the layers are of finite thickness. In theory, if a photon is not absorbed it will travel for an infinite distance within the particulate system.

1.4.3.3 Optical Penetration Depth

The optical penetration can be defined as the depth δ, at which the intensity of the radiation inside the material I falls to 1/e (about 37%) of the value of the incident beam, I_0. For a semi-infinite medium, in the range of validity of the K-M theory, the intensity at a distance z from the surface can be approximated[2)] as:

$$\delta = \frac{1}{\sqrt{K(K+2S)}} \tag{1.8}$$

2) D. Oelkrug, B. Boldrini, and R. W. Kessler, unpublished results.

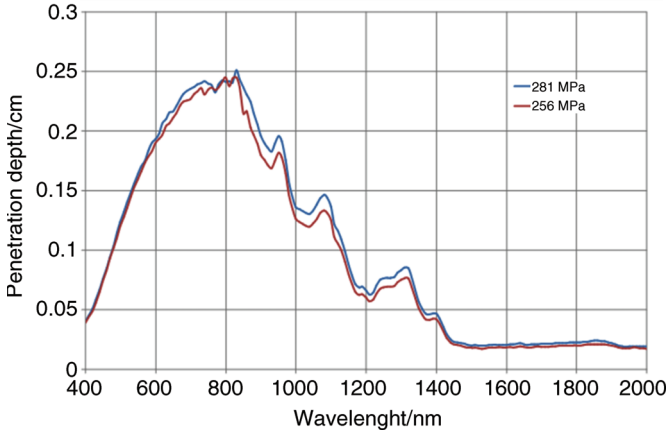


Figure 1.28 Penetration spectrum for the theophyllin tablet in cellactose at different degrees of compaction.

This is a generalized form of the penetration depth integrating absorption and scattering. Details will be described elsewhere.

Figure 1.28 shows the result of a calculated penetration spectrum of the theophyllin tablet example shown in Figure 1.4 at two different compactions. Because of the high absorbance at longer wavelengths the penetration depth is low. On the other hand, due to the strong absorption, the spatial resolution at higher wavelengths may be higher because the number of scattered photons is lower and specular reflectance has a lower probability. In the short NIR range (below 1100 nm), high penetration takes place, mainly due to the low absorption and mean scattering properties of the sample. As demonstrated, the penetration depth and, therefore, the scale of scrutiny can be determined, when the scatter and absorption coefficients are known. Similar approaches with similar results are described in [53, 57].

A much simpler approach is described in [58, 59]. Here the spectral response of an absorber is measured when this absorber is covered with different layers of scattering particles. The description of the determined path length is, however, strongly dependent on the geometrical set-up of the system, the sensitivity of the instrument and the properties of the particulates and, therefore, cannot be generalized.

1.4.4
Spectral Imaging and Multipoint Spectroscopy

Spectral or chemical imaging combines the physical and chemical characterization of samples by spatially resolved spectroscopy. The techniques may either be classified according to the used wavelength ranges or, more generally, into mapping or imaging techniques. *Mapping* usually means to get an image by exploiting the full spectrum (λ) of a local point through point measurements (at spatial x and y coordinates). The term *imaging* is used, when two-dimensional pictures (x, y) are obtained by a camera

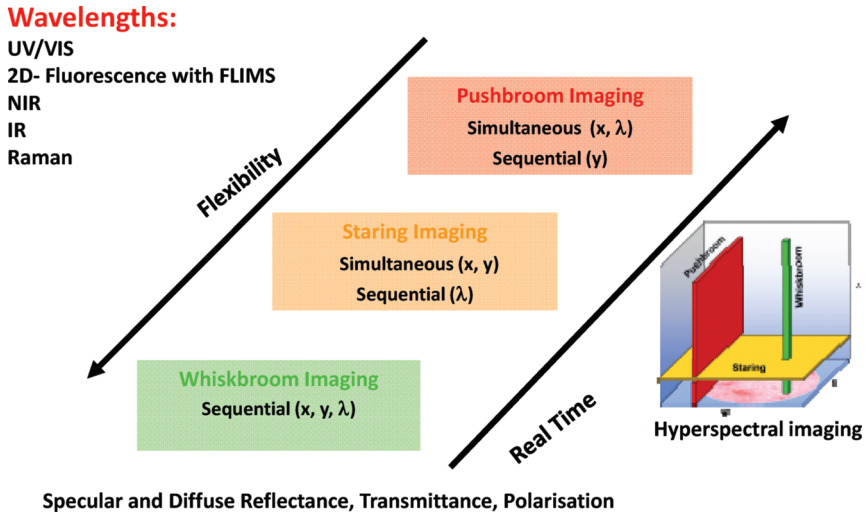


Figure 1.29 Visualization of the different imaging technologies: whiskbroom imaging, staring imaging, pushbroom imaging.

at different wavelengths (λ). In addition, *line scans* are defined as measured spectra along a line [60]. A more straightforward taxonomy is used in remote sensing. The different applied technologies are labeled as:

- Whiskbroom imaging
- Staring ("stardown") imaging
- Pushbroom imaging

Figure 1.29 visualizes the different techniques. All techniques can use the full optical wavelength range from the UV to the IR as well as Raman and fluorescence.

In process control, the spectroscopic method is often applied to moving samples and, therefore, should be as fast as possible. Hence, in this case the pushbroom acquisition mode is usually the best suited technique. Along a line of the sample, which represents the first spatial direction (x-axis), several spectra are measured at the same time. The pixel spatial resolution of this x-axis is determined by the number of pixels used and the distance of the sample from the spectrometer objective lens. The pixel resolution of the spectral λ-direction (z-axis,) is determined by the spectral range of the spectrometer (camera) and the number of pixels that are combined together to yield one spectrum. The second spatial dimension (y-axis) is correlated with time. If the sample is moved before the next line-scan starts, the time and the second spatial dimension on the sample (y-axis) are correlated to the moving speed of the sample. The data cube of the measurement thus represents the two spatial axes and the wavelength axis.

Figure 1.30 shows an example of the data cube of a hyperspectral image of a wood chip on which the letters RRI (Reutlingen Research Institute) are written with a UF (urea formaldehyde) resin binder that is commonly used in the production of

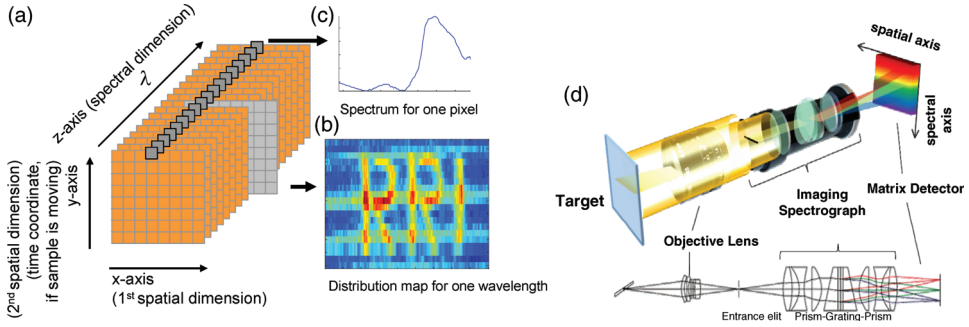


Figure 1.30 left: Sketch of three-dimensional hypercube (a) and distribution map for one selected wavelength $\lambda = 1506$ nm (b) and one spectrum along the spectral direction for one pixel $x = 90$, $y = 10$ (c). (d) Schematic drawing of a pushbroom imager (courtesy of Specim, Finland).

oriented strand boards (OSB). The objective of the chemometric treatment is to separate the information "wood chip" from the information "binder" and is intended finally to determine the thickness of the binder layer. A hyperspectral image was recorded for this sample with a pushbroom imager from Specim Finland. The first spatial resolution (x-axis) contains 180 pixels and each pixel represents 0.1 mm on the sample. For the second spatial direction (y-axis) 20 line-scans were performed after positioning the sample on a conveyer belt another 0.5 mm further in the y-direction from the starting position. This means 20 pixels for this y-direction with a spatial pixel resolution of 0.5 mm. The chemical information for each pixel is contained in 214 NIR absorption values in the wavelength range from 900 to 1700 nm (spectral pixel resolution is 3.7-nm). The resulting three-dimensional data cube ($180 \times 20 \times 214$) is sketched in Figure 1.30 together with a distribution map for one selected wavelength (1506 nm) and one spectrum along the spectral direction for pixel $x = 90$, $y = 10$. The full hyperspectral image has ($180 \times 20 = 3600$) spectra with 214 spectral readings in each spectrum.

To get the full chemical information out of the hyperspectral image it is necessary to look at the full spectral information contained in the image. This can be done by applying multivariate data analysis methodologies to extract the relevant information and at the same time reduce the dimensionality of the data. For images it is called multivariate image analysis (MIA) and for a qualitative analysis it is very often linked to principal component analysis (PCA). If a quantitative value for a quality or process parameter is required it is called multivariate image regression (MIR) and is mostly related to the partial least squares algorithms (PLS) [61].

When an optical fiber bundle is coupled to a pushbroom imager, multi-point spectroscopy at different locations in a reactor will be possible. These fibers can, however, also be coupled to various probes, thereby enabling simultaneous measurements like reflection, transmission or ATR measurements (see Section 6.2).

Pushbroom imaging technology is, for example, used to identify plastics in waste management systems. Figure 1.31 shows an example of a commercial application in separation of the different polymers automatically on a conveyor belt.

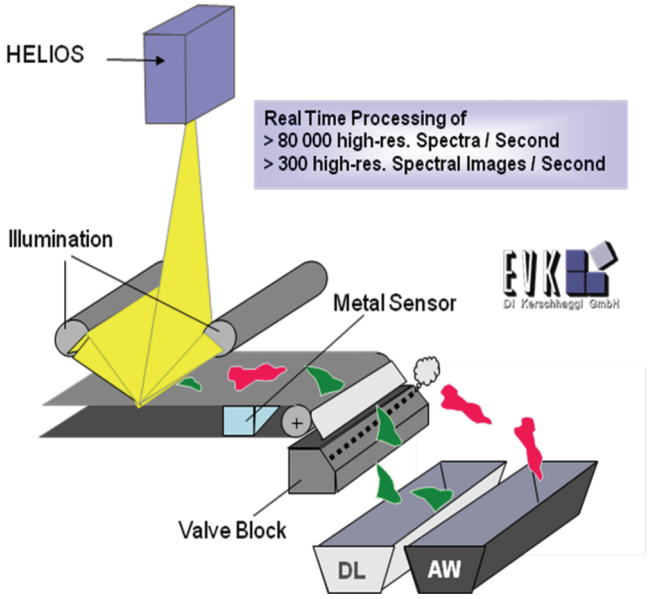


Figure 1.31 Real time characterization of polymers in plastic waste separation (courtesy of EVK company).

1.5
Survey Through Industrial Applications

1.5.1
Selection of Applications

This short survey through selected industrial applications of process analytical technology in the industry should illustrate the potential and widespread use that such methods already experience in the current industrial landscape. It should stimulate interest in potential problem solutions and with several thousand publications on the subject only in the course of the last five years is by far not intended to be comprehensive.

The objective of this section is to give also a short overview of the different applications of hyperspectral imaging in industry and science. It should provide some ideas on the heritage of information which is gained from hyperspectral images. Historically, the pharmaceutical industry uses mainly staring imaging technology, whereas food and agriculture is more focused on pushbroom imaging technology due to its background in remote sensing. Whiskbroom imaging technology will not be included in the selected applications as this technique is more restricted to scientific interests [62].

It is by far beyond the scope of this chapter to mention all relevant techniques and methods in detail, in the following sections only a brief summary of important applications of the various optical spectroscopic techniques is given. For an in-depth

summary of new developments in PAT the reader is referred to the review articles on process analytical chemistry by Workman and coworkers, for instance Refs [2, 63] which are published every couple of years and reflect the most important new developments in the field. Recent accounts of the state of the art of process analytical technology are also to be found, for example, in [6, 33, 64, 65].

1.5.2 Pharmaceutical Industry

The most important applications of process analytical technology in the pharmaceutical industry cover on-line measurements in raw material control, reaction monitoring and control, crystallization monitoring and control, drying, milling, cleaning validation, granulation and blending, drug polymorphism detection, particle size, moisture content determination, and tablet analysis, as well as packaging.

1.5.2.1 UV/Vis Spectroscopy

Spectroscopic analysis in the pharmaceutical industry is very often concerned with the analysis of drug formulations such as tablets. For instance, dissolution testing of tablets is mandatory and there exist guidelines of how to perform these tests. Diode-array spectrometer-based UV/Vis techniques can be employed in this context to monitor the dissolution behavior of tablets [66]. Another important application of UV/Vis spectroscopy is the controlling of cleaning operations. The cleanliness of vessels intended for pharmaceuticals, beverage or food production has to be guaranteed when production shifts from one batch to another with an intermittent cleaning step. Fiber-optic UV/Vis spectrometers have been used for this purpose. UV/Vis spectroscopy has also been used to determine the active ingredients content in tablets, for example paracetamol, ibuprofen, and caffeine [67].

1.5.2.2 NIR Spectroscopy

NIR spectroscopy is an established measurement technology that has been employed for several decades under *in situ* conditions. In comparison to the chemical industry, the diversity of industrial operations is much smaller. Hence, a number of standard applications of NIR spectroscopy have been developed that are established and widely used by numerous pharmaceutical companies. Typical standard applications are, for example, the NIR spectroscopic control of raw material identity by using fingerprint analysis of the incoming substances, the NIR monitoring of mixing processes, the NIR monitoring of granulation, or the NIR analysis of tablet formation and compaction. While raw material control in many cases is accomplished by laboratory equipment, in-process applications are of importance, especially in the control of the granulation process. An important target signal here is the time-dependent moisture content of the aggregate in the course of particle compaction. Besides monitoring the kinetics of the granulate water content, the kinetics of changes in the particle size distribution can be determined [68]. NIR spectroscopy is also used in the characterization of the mixture homogeneity of powder preparations. Here, two major strategies have been followed: (i) on-line NIR monitoring of the mixing process [69–72] by implementing either

stationary or moving NIR-probes directly in the mixing machinery, and (ii) imaging approaches, which will be described below. In the NIR spectroscopic analysis of tablets, the recent development of very rapid measurement devices based on diode array spectrometers allows 100% control of all manufactured tablets [73, 74], which represents an important step forward from random sampling. A typical problem in tablet manufacturing is not only the absolute amount of active ingredient but also its distribution within the particular formulation. While chromatographic analysis upon homogenization of the tablet by dissolution reveals the actual overall content of the drug in the tablet, the information generated by spectroscopic measurements is more complicated to unravel. Since NIR spectroscopy of tablets is typically carried out in diffuse reflectance the simultaneous contributions of scattering and absorption phenomena have to be considered and the arguments presented in Section 4.3 need to taken into account. Besides photon diffusion spectroscopy, here, too, imaging methods will play an increasingly important role in process analysis. Besides the active ingredient content and its distribution, in the automated analysis and quality control of tablets, physical parameters such as mass, diameter, thickness and hardness of the tablets are targeted [75, 76]. More examples on the application of NIR spectroscopy in combination with chemometric techniques can be found in the recently published review by Roggo [77] and in [78].

1.5.2.3 **Raman Spectroscopy**

Since interpretation of Raman bands is much more straightforward than qualitative interpretation of NIR bands and, hence, with Raman less chemometric effort is required, information deduction from Raman spectroscopy provides great potential also for the pharmaceutical industry. Monitoring of pharmaceutical blend homogeneity has been successfully accomplished using Raman based on a calibration-free approach by mean square difference between two sequential spectra; see, for example, [79]. In this study it was demonstrated that particle size and mixing speed significantly influence the time required to obtain homogenous mixing. Raman spectroscopy has also been used to follow the kinetics of polymorph conversion from one form to another [80]. Automated analysis of micro-titer plates can also be performed using Raman spectroscopy. Since it is possible to distinguish between crystal forms Raman spectroscopy has been suggested as a tool for high-throughput screening for different crystal structures [81]. Raman spectroscopy can also be used in combination with NIR data to determine correlations between physical pharmaceutical properties of tablets, such as hardness, porosity, and crushing strength [82]. Among others, the review published by Barnes [83] summarizes more recent examples of the application of Raman spectroscopy in the monitoring of pharmaceutical processes.

1.5.2.4 **Imaging Techniques**

There are still only a limited number of real life on-line applications for chemical imaging in process control in the pharmaceutical industry. Exhaustive reviews of different applications can be found in [33, 84]. Focus is mainly on three different uses: blend uniformity of powders and tablets, composition and morphological features of

coated tablets and granules, spatial changes during hydration, degradation and active release.

Pharmaceutical solid dosage forms are ideally suited for NIR chemical imaging. The systems are chemically complex and the distribution of the components affects dramatically the product quality and performance [85]. Recently, Lewis and coworkers compared wet granulation, direct compression and direct compression together with a micronized API. Besides the evaluation of the homogeneity of the mixing, they could also qualify the appearance and the morphology of API hot spots and agglomeration [86]. It is also possible to relate this information to the performance of the final product [87].

In another work, the utility of NIR chemical imaging in measuring density variations within compacts is demonstrated. The data are also used to relate these variations to tableting forces which are controlled by frictional properties and the quantity of the Mg stearate concentration during production [88]. This is possible as the spectral information also includes the percentage of specular reflected light at the surface of the tablet and the change in penetration depth of the photons which change after compaction.

Counterfeit pharmaceutical products are a real threat to the health of patients. NIR chemical imaging provides a rapid method for detecting and comparing suspected counterfeit products without sample preparation. The advantage of imaging is that the discrimination of the tablets is not only caused by changes in the chemical composition, but also from its spatial distribution. This reflects the use of different raw materials as well as the distribution of the components in the tablet which depends strongly on processing conditions [89]. Thus the combined chemical and morphological information provides an individual measure for the characterization of tablets.

Applications of chemical imaging in the pharmaceutical industry are mainly related to NIR imaging. Some papers describe also the use of terahertz imaging. Terahertz is ideally suited to the identification of chemical components with strong phonon bands, thus components with high crystallinity can easily be distinguished from amorphous systems [90].

1.5.3
Food and Agriculture

Spectroscopic quality monitoring, and especially NIR spectroscopy, has a very long tradition in the food and agricultural industries. The main areas of application here regard the quality control of raw materials, intermediate products and final products. Many different sample types have to be dealt with, as reflected by the vast diversity of possible food stuff, ranging from liquids, powders, slurries to solid materials. One major problem often encountered in the application of NIR in food technology is the preparation of appropriate reference materials – soft matter such as food usually is connected with complex and varying matrix systems. Typical applications include the transmission, transflection and diffuse reflection measurement of crop grains and seeds, fruits and vegetables, livestock products, beverages, marine foods and processed foods.

1.5.3.1 UV/Vis Spectroscopy

UV/Vis spectroscopy is still an under-represented technique in process analysis. The advantages of UV/Vis spectroscopy are the low cost and high reproducibility of the equipment and its precision, especially in quantitative analysis. In recent years, UV/Vis analysis has been successfully implemented in the beverage industry and for wine color analysis [91–93]. The main application is the use of color cameras in food processing, which is also a sort of spectroscopic application.

1.5.3.2 NIR Spectroscopy

Water is an important issue in the food industry and can be measured using NIR spectrometers integrated in the food processing facility [94]. Typically, a rotating filter wheel is used and the characteristic absorbance peak of water at 1940 nm is used for calibration. By this method, water has been determined in a wide variety of dry products, such as instant coffee powder, potato chips, bonbons, tobacco, wheat, noodles, cookies, or dry milk powder [95]. Another important food constituent is fat, which may also be measured using infrared spectroscopy; in aqueous systems, however, it is preferable to use measurements in the mid-infrared range to avoid interfering water bands [96].

Although determination of major components in foodstuff, such as acid or sugar content, is still an important task, increasing attempts are made to determine complex quality parameters governing the expected sensory perception or storage stability of a certain product instead of only measuring one defined component. For comprehensive and recent reviews on the subject, see, for instance [97–101].

Assuring food safety is still a key issue in the food industry. Bacteria usually show broad bands in the NIR region even on taxonomically unrelated bacteria. However, using MVA it is possible to differentiate most of the bacteria [102].

1.5.3.3 Raman Spectroscopy

Raman spectroscopy is also a versatile method in applications related to food and agricultural products [103]. Food components, such as carbohydrates, edible oils, cyanogenic components, or proteins can readily be identified due to their characteristic absorption pattern by means of univariate characterization and peak assignment. For instance, proteins can be studied by comparing changes in the amide I (carbonyl stretch) band, which shifts from 1655 cm^{-1} for the α-helix to 1670 cm^{-1} for the anti-parallel β-sheet structures and, hence, allows detailed studies of food stuff. Cyanides show a very distinctive sharp band for the $C \equiv N$ triple bond at 2242 cm^{-1}. Inorganic food constituents, such as calcium carbonate, can also be identified due to their very sharp spectra. It was, for instance, possible to characterize the pre-freezing treatments of shrimp by studying the relative differences in Raman signals obtained from different crystal structures of calcium carbonate (ratio between calcite and vaterite), respectively, by studying the dehydration of the hexahydrate (ikaite, absorbance at 1070 cm^{-1}) to the anhydrous form (vaterite, 1089 and 1075 cm^{-1}).

Certainly, the potential of Raman spectroscopy can be enhanced by subjecting the spectral information to chemometric post-treatment, opening the field for rather

quantitative applications of Raman methods. For example, alginate powders have been studied quantitatively with respect to their characteristic β-D-mannuronic acid to α-L-guluronic acid (M : G) ratio. It is recognized that Raman spectroscopy must no longer be regarded as an exotic tool for routine analysis of soft matter like food but has its rightful place in the PAT toolbox available for the food and agricultural industries [103].

1.5.3.4 Imaging Techniques

On-line chemical imaging in agriculture is mainly remote sensing. Satellite or aerial remote sensing (RS) technology uses nowadays pushbroom imaging technology in the Vis, s-NIR and NIR-range. Vegetation images show crop growth from planting through to harvest, changes as the season progresses and abnormalities such as weed patches, soil compaction, watering problems, and so on. This information can help the farmer make informed decisions about the most feasible solution.

The differences in leaf colors, textures, shapes, or even how the leaves are attached to plants, determine how much energy will be reflected, absorbed or transmitted. The relationship between reflected, absorbed and transmitted energy is used to determine spectral signatures of individual plants. Spectral signatures are unique to plant species [104].

Remote sensing is used to identify stressed areas in fields by first establishing the spectral signatures of healthy plants. For example, stressed sugar beets have a higher reflectance value in the visible region of the spectrum from 400–700 nm. This pattern is reversed for stressed sugar beets in the non-visible range from about 750–1200 nm. The visible pattern is repeated in the higher reflectance range from about 1300–2400 nm. Interpreting the reflectance values at various wavelengths of energy can be used to assess crop health. The comparison of the reflectance values at different wavelengths, called a vegetative index, is commonly used to determine plant vigor (for details, see [104]).

In the food industry, numerous on-line controls are still made by human vision, especially for sorting bad looking products. Cameras can perform this task more efficiently and, using RGB cameras, a limited certain spectral differentiation is possible in machine vision [105]. However, due to the great variety of states (solid, fragmented, etc.) of the food, shapes, color and chemical composition, as well as seasonal variations it is difficult to monitor and control food in an unbiased manner. The driving force to introduce NIR imaging was to qualify food not only on its appearance but also on its chemical composition, such as water or starch content. An extensive demonstration of different applications is given in [105], which includes on-line characterization of chemical composition, detection of external contamination, surface and subsurface nonconformities and defects in food.

The airborne or on-line information for process control in food qualification can be complemented with a higher resolution by hyperspectral imaging techniques on a laboratory scale. The ripeness of tomatoes was qualified using Vis-spectroscopy [106], moreover, apples were qualified on the quantification of starch and other chemical compounds [107, 108]. Differences in phenol-typing in plant breeding development can also be visualized by NIR imaging as shown in [105].

Chemical imaging in food and agriculture can also be used to identify diseases, rot and contamination by insects, for example, larvae. A method to detect animal proteins in feed is described in [108]. The objective was to determine the limits of detection, specificity and reproducibility.

1.5.4 Polymers

Process analytics is even more widespread in the chemical industry and such diverse processes as polymerization, halogenation, calcination, hydrolysis, oxidation, corrosion, purification, or waste disposal, to name just a few, can be advantageously monitored using PAT tools. As an example for the chemical industry, a few application examples from polymer manufacturing are given.

1.5.4.1 UV/Vis Spectroscopy

Applications of UV/Vis spectroscopy in the polymer industry are numerous; for example it can be successfully used to determine the color of extruded plastics [109]. Color is a key quality criterion for the customer and automated on-line analysis of color is of great importance and relatively easily accomplished, and various experimental set-ups have been described. Another example of the industrial application of UV/Vis spectroscopy is determination of the thickness of polymer films.

1.5.4.2 NIR Spectroscopy

NIR spectroscopy is widely used in laboratory and industrial applications for material classification [65]. One of the most important processes in the industrial synthesis of polymeric materials is polymer extrusion. NIR has been employed to control the extrusion process, for example in terms of characterization of the starting formulation and of the final polymeric product [110–112]. Moreover, the additive content of polymer blends can be determined by using NIR [113, 114]. For that kind of measurement, on-line probes can be installed before or after the actual extrusion unit. More sophisticated application of NIR process analytical technology uses the direct implementation of NIR sensors in the extrusion machinery. Here, a flow cell with an integrated NIR sensor may be located after the mixing unit, directly before the hot polymer melt enters the mold, and spectra may be recorded either in transmission or diffuse reflectance, depending on the transparency of the polymer. Since the absorbance of the glass fiber optics has been shown to be temperature dependent [115], the temperature needs to be carefully controlled in the measurement chamber and the measured spectra have to be baseline corrected. Important requirements for reliable extrusion monitoring are reproducibility, accuracy and long-term stability of the NIR system [116]. For each measurement position (for example, at the end of the extruder, in the transport zone of the extruder, or at the cooling, extruded polymer material) separate calibration has to be performed that, besides polymer composition, also includes parameters like color or particle content and particle size distribution [117].

The moisture content in polymers has also been determined by NIR spectroscopy [116]. Besides composition, physical parameters of polymers such as density [118] and melting flow index [119] have been determined by NIR, and even more complex rheological parameters have been correlated with NIR spectra [120]. When NIR spectroscopy was used in combination with an ultrasonic treatment, even the bubble formation in the transformation of polystyrene was observed, which demonstrated the versatility of the method [121]. A review of process analytical applications in the polymer industry has recently been published by Becker [116]. Siesler also gives a very good overview of the potential of NIR in polymer analysis [122].

1.5.4.3 **Raman Spectroscopy**

Raman spectroscopy has been shown to have great potential in polymer production, for example for monitoring the emulsion polymerization of various polymers [123–126] or polymer curing reactions [127, 128], and also for the determination of the residence time of TiO_2-containing polypropylene in an extruder [129]. Polymer films can also be characterized in real-time during the blown film extrusion process with respect to composition, crystallinity and their microstructure development [130]. Besides polymer bulk and film characterization, application of Raman spectroscopy as a PAT tool may also be very promising in the characterization of synthetic and natural polymer fibers and their composites [131], foams [132], and even liquid crystals [133].

However, so far not so many applications of Raman spectroscopy in industrial polymer processing have been described in comparison to NIR applications. NIR spectroscopy is by far the most commonly employed method here. One major reason for this is the much higher cost of Raman equipment. Recent developments towards lower investment costs will certainly broaden the scope of potential applications.

1.5.4.4 **Imaging Techniques**

While standard spectrometers only allow measurement at one sampling point at a time, NIR spectral imaging techniques can identify, in real-time, both the size and shape of an object, as well as the material it is made from. The robust classification of materials, such as polymers, is based on their characteristic reflectance spectra. Sorting, for example, requires the correct material, size and shape of the entire object to be known for reliable separation [134–136].

1.6 Perspectives

1.6.1 Technology Roadmap 2015 +

Regarding the various PAT, the following trends in future development of process analytical equipment have been identified and summarized in the roadmap 2015 + for process sensors published by NAMUR/GMA [137].

1) It is expected that sensor systems will display much greater robustness and long-time stability, that is, they will be required to depend on much less efforts in maintenance and, hence, will have a significantly lower cost-of-ownership
2) New in-process sensors will not only be incorporated in the design of newly erected industrial plants but they will also be employed in the optimization of existing processes
3) The new requirements for process analysis systems will not be exhausted by the pure collection and storage of process data. Information on the current state of intermediates while production is still operating and predictions on expected end-product properties will be increasingly used for controlling and process management purposes.
4) For specific purposes, an increased accuracy of the gathered process information will be required
5) As a most important trend, spatially resolved information of process and material parameters will become of increasing importance.
6) Since interfaces play an important role for practically all industrial and engineering areas, the localization, identification and characterization of interfaces will play an increasingly important role in process analysis applications
7) There is a growing trend toward manufacturing products in an environmentally compatible way. This means that a growing number of synthetic processes currently performed in a classic chemical pathway will be translated into biotechnological approaches. As a consequence, process monitoring systems suitable for biotechnological applications, and specifically fermentation processes, will gain in importance and will have to be further developed and refined
8) In bioprocess technology, an industrially feasible and robust in-process analysis of specific targeted proteins would be a revolutionary achievement.
9) In order to comply with an ever increasing cost-pressure imposed on industrial processes, the substitution of sophisticated and expensive process analysis methods carrying a significant cost of ownership during maintenance by low-cost, ideally single-use devices that are disposed of after having delivered the required information is an important task for future research and development activities in the field of process analyzers.
10) The growing use of renewable resources or recycled materials as environmentally compatible starting materials for new products imposes novel analytical problems on process analytical devices which will have to be addressed in future development and applications of process analysis
11) The sensitivity of process analysis devices in many cases will have to be significantly increased. For instance, in the analysis of potentially harmful or catalytically effective trace components in gases, continuously lower detection limits will be required
12) In the context of a holistic process management, there is also an increasing demand in developing non-invasive analysis techniques in the field of warehouse logistics and product organization

13) An overall trend towards in-line measurements is evident, which in turn means that intensification of research on the translation from laboratory analytical methods into stable, processable and robust in-line methods that can routinely be applied will be required

Some of the new challenges and developments in PAT are discussed in the following sections.

1.6.2 Medical Applications and Tomographic Imaging

The next step in imaging is to integrate the *z*-spatial axis into the data cube. Especially for medical applications, it is important to feature the spatial distribution in all three directions.

Techniques which are available for 3D-imaging often lack the spectral information or are difficult to introduce into in-line or high throughput applications like optical coherent tomography (OCT) and laser scanning confocal microscopy. As stated in the introduction, medical ultrasonography, magnetic resonance imaging (MRI) and confocal microscopy are not suited to morphological or spectral imaging: the first two have poor resolution; the last lacks millimeter penetration depth and suitable applications in process control. On the other hand, in process analysis, electrical resistance tomography (ERT) is very popular nowadays and involves measurements around the periphery of an object for example, a process vessel or a patient. The instrument operates by taking data from an array of electrodes in contact with the process media. The electrical field lines "communicate" and are, therefore, affected by the presence of conducting and nonconducting regions [138]. However this technique does not provide any information on a molecular basis.

New developments in laser scanning confocal microscopy allow the use of different lasers at selected wavelengths; thus spectral imaging may then be realized. Also, when fluorescence is excited during scanning, for example, acousto-optical tuneable filters can be used to analyze the fluorescence spectrum at each focal point. However, there is still a long way to go for on-line or at-line applications in process control. This is also true for fluorescence imaging techniques, including lifetime imaging systems without using fluorescent markers [139]. On the other hand, multiphoton tomography is now at the edge of a broad "at-line" application in clinical high throughput *in vivo* studies [140].

Diffuse optical imaging (DOI) is a new emerging technique for functional imaging of biological tissues. It involves generating images using measurements in the visible or s-NIR-light scattered across large and thick tissues (about several centimeters) [141, 142]. As shown in Figure 1.7, penetration depth in the s-NIR range is high, therefore the objective of DOI is to provide low cost sensors for *in vivo* applications including imaging for example, of breast and brain cancer. The key issue, however, is the image reconstruction which is difficult due to the scattering of the material and, therefore, low spatial information of the data. Progress has been made on developing

more realistic and efficient models of light transport in tissue and solving the *ill-posed inverse* problem in an increasingly rigorous way [141].

Another way to solve the diffusion of photons problem and to localize spatially resolved features is time-resolved NIR-spectroscopy. This technique enables the separation of the absorption properties of the sample from the scattering properties. This improves the localization of changes in the physical parameters, for example, in a tablet [143].

Until recently, researchers did not extensively explore the material interactions occurring in the terahertz spectral region, the wavelengths that lie between 30 μm and 1 mm. Terahertz spectroscopy can be used to image through materials yielding high spatial resolution in the *x*, *y* and *z* directions. The technique also has the ability to resolve both time and amplitude information. Terahertz has the potential to be applied to many applications, including: on-line non-destructive testing for quality control of products and packaging for industrial markets; weapons and explosives detection for homeland security and defense markets; topical imaging for the medical market; quality control of external skins on space vehicles for the aerospace and military markets and many more. The technique is still quite expensive but will find its way into process control in the future [144].

1.6.3
Multi- Point-Information Systems in Manufacturing

The main objective of any process control is to guarantee a constant and specified product quality. Especially, maximum yield at a minimum of manufacturing costs are more and more important parameters, even in the pharmaceutical industry. Complex manufacturing with many different successive production steps must introduce a feed-back and a feed-forward control in the future. This requires specific efforts to integrate at each step of the production line an appropriate quality control for example, by spectroscopic methods.

Instead of using a single spectrometer at each individual production step, pushbroom imaging systems with numerous attached fiber bundles allow individual control of the quality at each intermediate and the final step. In this context, the pushbroom imager is used as a multipoint information source and can substitute a moving multiplexer. Many fibers per spectrometer can be used for simultaneous measurements. In addition, different spectrometer technologies for UV–Vis–NIR, fluorescence or Raman can also be combined. Thereby the probe becomes a multi-information system, which describes the sample in an ideal and complete way.

Figure 1.32 shows a schematic diagram of some production steps in tablet production with the integration of a multipoint spectroscopic information system using push-broom imaging technology.

In this example the pushbroom technology is able to substitute several single spectrometer systems. For example, at the fluid bed dryer, the moisture or the homogeneity of the blending process is controlled on a molecular level. Combined with multivariate data analysis science-based production can be realized.

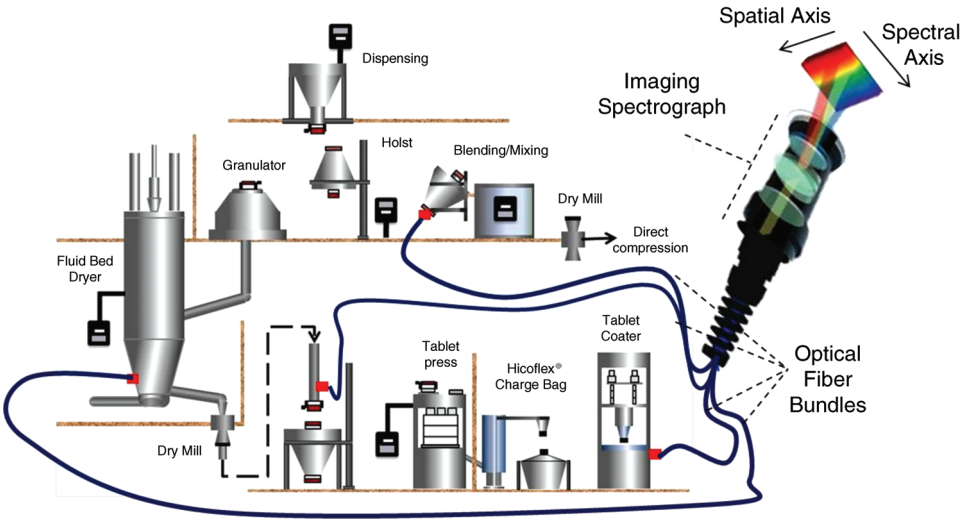


Figure 1.32 Schematic diagram of a tablet production process with different possibilities for pushbroom imaging.

1.6.4 Multimodal Spatially Resolved Spectroscopy

In recent years multimodal methods have matured to an accepted new technology, especially in the field of medicine [145]. The term multimodal relates to the possibility of collecting information on chemical and physical parameters at the same time. Not only chemical changes at the molecular level but also morphological changes in the sample can be detected. Complex interrelations of the system can be reduced and described in an easy and complete way. Several different imaging techniques are frequently used to realize the multimodal concept.

Process control is subject to a continuous change and the approach of multimodal methods will be extended in the future. The increasing complexity of high quality products and the cost pressure demand new strategies. In the future, the focus will not only be on process optimization but also on tailoring property profiles of products according to specifications and individual desires of customers. This will allow the creation of operating instructions, including the choice of materials and process parameters, to design a product with a given preference profile. Multimodal methods will then also enable the realization of multipurpose optimization [146].

Optical molecular spectroscopy, and in particular chemical imaging, will play an important role in implementing these objectives. By combining different wavelength ranges and techniques multimodal spectroscopy synergistically provides much more useful information than each technique on its own. It produces complementary chemical and morphological information about reaction products. Measurements can be carried out quickly, sensitively, selectively and economically at a reasonable

price. In principle, the implementation of the multimodal approach can be achieved in three different ways.

The first implementation strategy uses different wavelength ranges. The UV range is more suitable for scattering effects, while the short near-infrared range has a much higher penetration depth because of the low absorption probability in organic samples. Fluorescence techniques for excitation and emission spectroscopy can be used to separate molecules due to their different spectral signatures. Raman spectroscopy as a light scattering technique can be used as a fingerprint method to identify molecular structures or bonding effects.

The second implementation strategy involves using different technologies within the same wavelength ranges. For example, diffuse reflectance and transmission in the UV or NIR can be used to separate morphological scattering from chemical effects [9]. In addition attenuated total reflection (ATR) spectroscopy can be used to analyze highly concentrated samples without prior dilution and avoiding interference by scatter.

The third implementation strategy deals with laterally resolved measurements to achieve the desired differentiation. Angular resolved spectral measurements or line scans with a pushbroom imaging system lead to different penetration depths, which are highly specific for particulate systems. Besides the chemical information, parameters like homogeneity, particle size, particle distribution and density can also be detected.

1.6.5
Microreactor Control and Reaction Tomography

Microreactor technology is a powerful tool and has become indispensable over a wide application range from organic synthesis to enzymatic controlled reactions [147, 148]. A miniaturization with the possibility of reaction tomography, and thus a significant reduction in costs, will be the next step in the foreseeable future. Many chemical reactions, especially organic and fine chemical synthesis, could already be transferred to continuous microreaction processes [149]. The small geometric dimensions result in an intensified mass and heat transfer which often leads to increased yield and selectivity compared to the classical batch approach. However, microreaction technology today is still at the threshold between academia and industry.

A crucial factor for the successful implementation of microstructured production processes in industry is a suitable process analytical technology. Time and spatially-resolved on-line analysis must be implemented directly in the microfluidic channels. Thus parallel and multiplexed measurement technologies are needed to reduce costs and increase the robustness of information. To date, no commercially available solutions exist.

Typical state of the art procedures to study chemical processes use flow cells positioned after the microstructured environment or off-line methods. However, these approaches have several disadvantages. A critical point is the creation of distorted results due to changed geometrical proportions with measurements

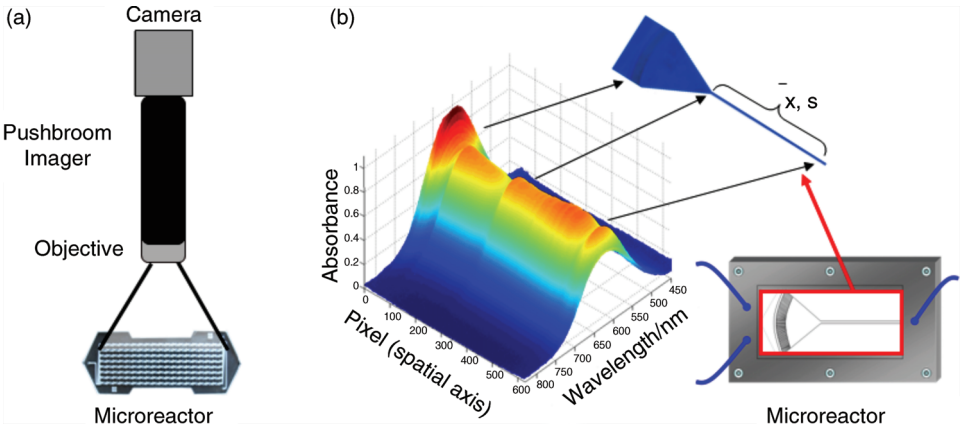


Figure 1.33 (a) Schematic diagram of the experimental set-up for an on-line spectrometer system. (b) Example of reaction monitoring of different concentration levels of an aqueous blue solution flowing through a microreactor system.

downstream of the microstructured environment. Moreover, no information about the actual reaction process inside the microreactor is generated. The major disadvantage, however, especially for industry, is the increasing costs for analytical devices required to assure constant product quality. The production of high added value chemicals by means of a microstructured process requires several hundred microreactors in order to produce sufficiently large amounts of final product. Therefore, large numbers of flow cells, each attached to a separate spectrometer (= pushbroom imager), are needed to meet these unique requirements.

An alternative technology is to analyze several microchannels simultaneously or to analyze a single microchannel spatially resolved along the reaction path using pushbroom imaging technology. This type of optical on-line spectroscopy is an ideal tool to characterize chemical reactions in a fast and reliable way, even on a molecular

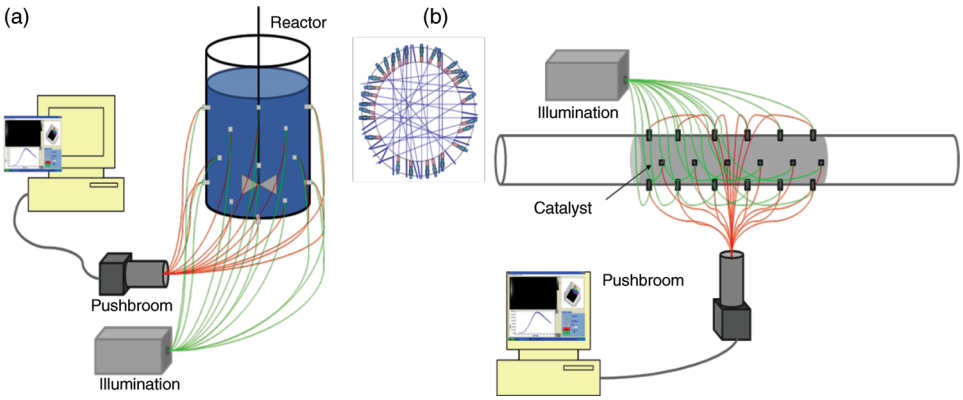


Figure 1.34 Pushbroom imager as a multipoint spectrograph for tomography of a batch reactor (a) and a continuous reactor (b).

level. Transmission or reflectance spectra are registered through a fixed prism–grating–prism optics with a two-dimensional CCD camera attached to it. Thereby, the *x*-axis of the CCD array corresponds to the spatial resolution and the *y*-axis of the camera provides the full spectrum of the sample. Figure 1.33 shows the experimental set-up for an on-line spectrometer system as well as an example for reaction monitoring.

Figure 1.34 shows a sketch of a pushbroom imager connected to a fiber bundle where a batch reactor can be analyzed, and another example is shown where spectroscopy is applied to a continuous reactor.

This multipoint spectroscopy can also be applied to microreaction systems to analyze along a reactor or as a multiplexed microreactor analyzing tool.

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