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Miniaturization in Analytical Chemistry

1.1 Introduction

Miniaturization is rapidly growing, with novel ideas emerging in recent years. Like other fields, analytical systems have been affected by this new technology. Concretely, the capacity to carry out laboratory operations on a small scale using miniaturized devices is very appealing. *Micro total analysis systems* (μTAS), also called *lab-on-a-chip*, have renewed interest in scaling laws in the last 10–15 years. A small scale reduces the time required to synthesize and analyze a product, as greater control of molecular interactions is achieved at the microscale level. In addition, reagent cost and the amount of chemical waste can be very much reduced.

Now, at the beginning of the twenty-first century, it is clear that the lab-on-a-chip approach is starting to be considered as a potential analytical tool in many application fields. Nevertheless, additional efforts must be addressed to two main points: (i) the laws at nanometre scale must be established, as basic physical and chemical fundaments cannot be applied; and (ii) more applications demonstrating the real use of these systems must be developed, particularly in the area of complex samples analysis. There is no doubt that miniaturized chemical analysis systems have a tremendous potential. For instance, it is foreseeable that such devices will allow the study and analysis of complex cellular processes, facilitate the development of new diagnostic abilities that could revolutionize medicine, and have applications in environmental monitoring, food analysis and industry.

Some miniaturized analytical systems, such as capillary gas chromatography, microliquid chromatography and microcapillary electrophoresis – which can be

Miniaturization of Analytical Systems: Principles, Designs and Applications Angel Rios, Alberto Escarpa and Bartolome Simonet

considered as intermediate levels of miniaturization – have been consolidated in routine laboratories for the analysis of complex samples. There is no doubt that the majority of real analysis requires an appropriate sample treatment step. In this way, important efforts have been made to reduce the sample volume and its feasible manipulation in a miniaturized environment. The development of flow-processing devices for analyte purification/preconcentration, both on-column and on-fibre solid phase microextraction, hollow-fibre liquid phase microextraction, sorptive stir bars and so on are clear examples of miniaturization of the sample treatment step. The direct coupling of these methodologies (using at-, in- or on-line modes) to the instruments even allows an additional reduction of sample volume. At the same time, these coupled approaches reduce human manipulation and increase the degree of integration of the different analytical steps. The extension of this concept results in the modern concept of miniaturization: a miniaturized instrument integrating sample input, pre- and post-column reaction chambers, separation columns and detection units on to a single and small device.

In this chapter, an overview of miniaturization in analytical science is presented. In addition to preliminary aspects dealing with miniaturization, other issues such as the classification and definition of terms, the features and main challenges of miniaturized analytical systems, the incidence of miniaturization in the different steps of the analytical process and present trends in miniaturization are covered in this chapter.

1.2 Miniaturization as One of the Critical Trends in Modern Analytical Chemistry

Modern analytical chemistry is more and more a scientific discipline connected to the real world. International standards require a certain quality of client service from analytical laboratories, such as the norms ISO 9001 and, more specifically, ISO 17025. Hence, laboratories are encouraged to obtain client feedback in order to identify their real information needs. As Figure 1.1 represents, the developments and trends of modern analytical chemistry must be coherent with this feedback. Trends toward simplification ('ease of implementation or use'), automation ('electro-mechanical self-operation', involving a feedback loop to control the system without human participation) and miniaturization ('small scale'; 'construction to a very small scale') are well recognized. A common interface between these three characteristics can be identified as an attractive area for interesting developments, bringing about the so-called (re)-evolution of analytical chemistry from the end of the twentieth century onwards. This trend has a strong influence on present analytical chemistry. On the other hand, a high degree of simplification and automation is intrinsically involved in miniaturized systems. The simplification, mechanization/automation and miniaturization of the analytical process must assure the fitness for purpose of the analytical information generated with respect to customer information needs.

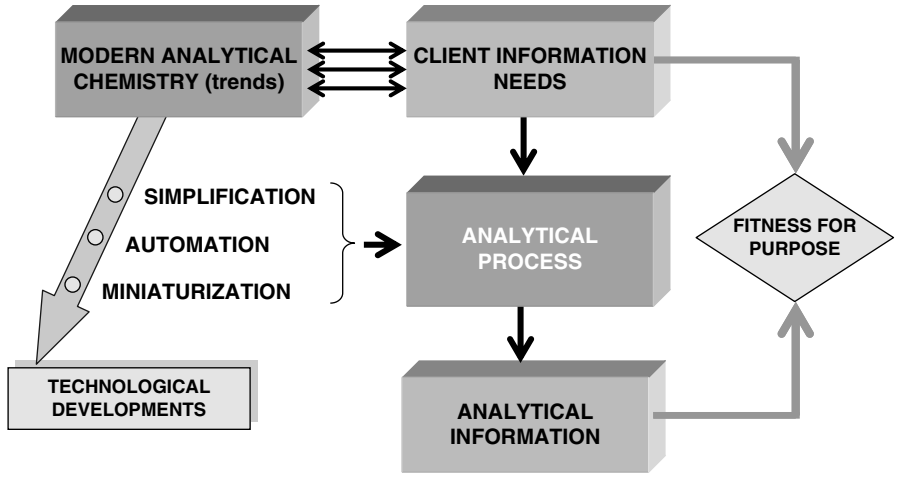

Figure 1.1 *General trends in modern analytical chemistry and their connection with the analytical process and client information needs*

Analytical microsystems, when used to provide this analytical information in analytical laboratories, are no exception (Chapter 10 deals with issues related to the performance and reliability of the information provided by analytical microsystems). Following the scheme shown in Figure 1.1 and the idea of implementing different degrees of miniaturization of the analytical process, Figure 1.2 shows the number of steps connecting the real world (the client information needs) with the final results generated by the application of the measurement process. In this figure,

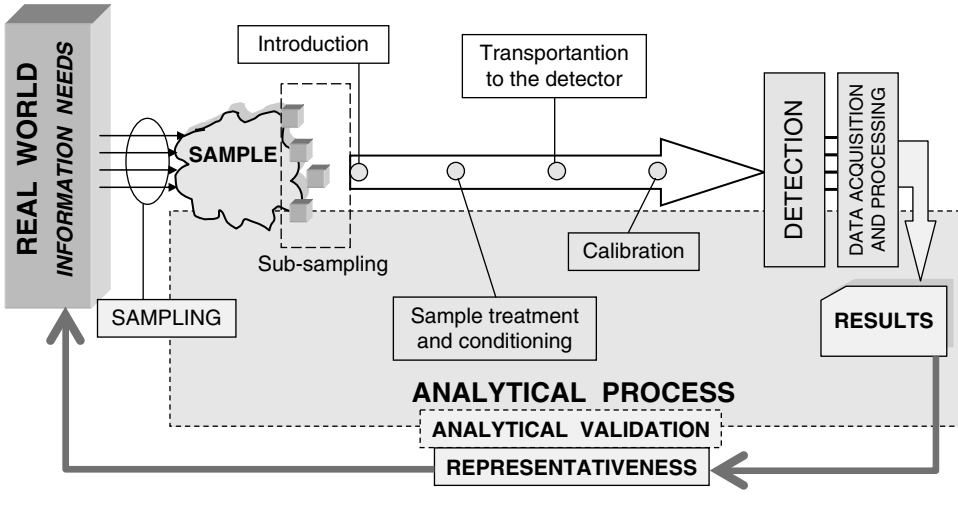

Figure 1.2 *Scheme of the analytical process noting the different steps and activities, with special attention to miniaturization aspects*

the main steps and activities requiring especial attention for miniaturization purposes have been identified. Thus, among these activities must be considered the sampling (and the corresponding subsampling), calibration and validation of the entire analytical process; steps such as introduction of samples, treatment and conditioning of samples, transportation to the detection point, measurement, and data acquisition and processing; as well as final goals such as quality and representativeness of the analytical results. The possibilities and difficulties inherent in the miniaturization of each of these steps and activities are quite different. Even the level of miniaturization is an important issue. By considering the entire analytical process (fortunately, miniaturization implies reduction or elimination of some of the mentioned steps), the main problems for miniaturization affect the preliminary operations (sampling–sample introduction and sample treatment steps). Conversely, detection and signal transduction, as well as data acquisition and processing, are steps which can achieve a high degree of miniaturization.

It seems clear that the final objective of miniaturized systems in the analytical domain is represented by μTAS [1]. In fact, ideally a TAS performs all the analytical steps (sample preparation, analyte separation and analyte detection) in an integrated instrument. The philosophy of TAS has been the enhancement of online and automated analysis, as well as of the analytical performance; however, significant drawbacks still exist, which should be the subject of future work in this field (for example, sample introduction and the successive analysis of a set of samples, slow sample transport, and the necessity of fabricating interfaces between the different components). The μTAS concept was developed from the modification of the TAS by downsizing and integrating its analytical multiple steps (sample preparation, separation and detection) on to single monolithic devices [1]. In essence, a μTAS is a device that improves the performance of an analysis by virtue of its reduced size. But not just analytical tasks can be performed in a miniaturized system; other chemical functions such as synthesis can also be performed. For this reason, today the μTAS concept is also known as 'lab-on-a-chip'. After almost two decades since the concept was introduced, now is a very exciting time for μTAS, due to the large bulk of advances and challenges that appeared in the last revision published in *Analytical Chemistry* [2].

Some guidelines may be stated concerning the approach of miniaturization as a whole:

(i) Miniaturization implies a micro-size environment. It is very important to keep in mind that miniaturization is not only a decreasing of scale, but that other forces and phenomena are present in micro-size environments [3–5].
(ii) Miniaturization requires technology facilities. Thus, to understand what the revolution of miniaturization in analytical chemistry means, it is necessary to consider the synergic *marriage* (relationship) between some part of the technology and the objectives of modern analytical chemistry. In other words,

in their fundamental sense, the objectives or demands of analytical chemistry should answer to some questions related to miniaturization, such as why, when and how miniaturization is performed. Obviously, the answer to the last question comes from the existing technology facilities [6]. Figure 1.3 shows the outlines of this complementation. From an analytical point of view, traditionally chemical analyses have been performed in central laboratories since they require skilled personnel and specialized analytical instrumentation. However, the trend today is to move chemical analyses close to the 'customer' or the bulk sample (*in-situ* analysis). Miniaturization plays a prominent role in the decentralization of chemical analysis. As a consequence of this, miniaturized analytical devices should be portable, easy to operate and reliable. The automation of chemical analysis also requires the real implementation of μTAS in analytical laboratories. In fact, analytical microsystems could ultimately be represented as a black box, where the role of the users basically consists in providing the samples and pushing a start button.

From a technological point of view, several potential benefits of analytical microsystems can be observed (see Figure 1.3): very well-understood knowledge about microfabrication techniques and the chemistry of materials; the possibility for fabricating sophisticated microcircuits according to analytical demands/objectives; and high compatibility with mass production. Another advantage lies in the possibility of opening new directions in microfluidics, such as the presence of laminar flow. On the other hand, one weak point of analytical microsystems comes

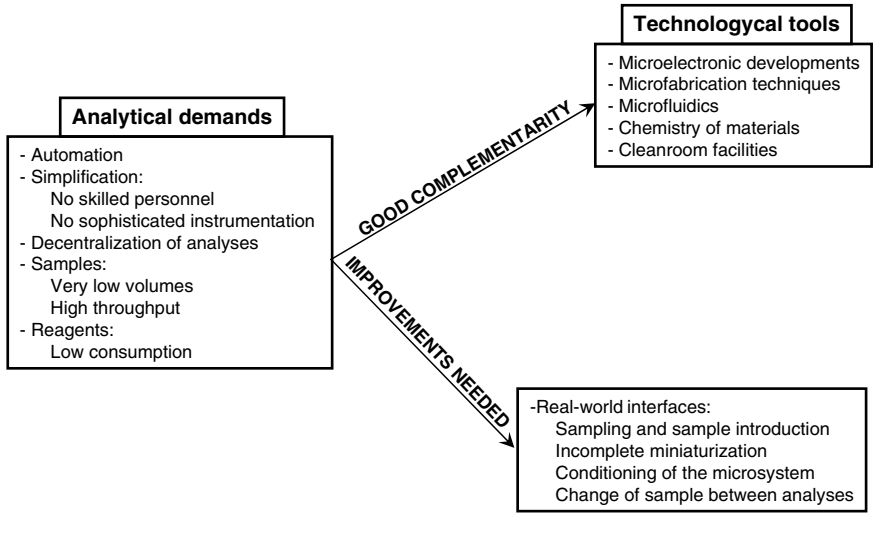

Figure 1.3 *Relationships between present analytical demands and technological developments for miniaturization*

from the present technology; sometimes the complete miniaturization of all the electronics and mechanical parts of the system is not allowed. Moreover, these elements are expensive and the required technology is not always available (for instance, cleanroom facilities).

The microtechnology used in the miniaturized environments could also be understood as the integration between the possibilities offered by microfabrication and microfluidics. Both microfabrication (micromachining) and microfluidics are inversely connected with the grade of complexity in the analytical chemistry process. Indeed, while microfabrication plays the prominent role in the separation and detection systems, the physical control of the process has a prominent role in all steps of sample preparation. In addition, the required microfabrication is very sophisticated in the sample preparation step. Chapter 2 deals with the tools for designing miniaturized analytical systems.

Two other intrinsic characteristics of analytical microsystems have clear connections with technology developments: the extremely low sample volumes used, and the presence of laminar flows. Small volumes of both sample and reagent (pl–nl levels) are representative of most miniaturized systems. This characteristic has clear advantages associated with cost and analytical throughput, but it also presents disadvantages, such as the suitability of detection techniques. Consequently, much research effort has been focused on the development of miniaturized and sensitive detection units [7], and today the detection improvements are still one of the most important research focuses [8]. As a consequence of the low volumes required, a very precise handling of sample is crucial in microanalytical systems, and a high dependence of the surface properties of microchannel manifolds and interconnections and dead volumes is observed [3]. Complex fluid manipulation at femtolitre and nanolitre scales is readily achieved without any mechanical valves or external pumps by using the electrokinetic phenomena [9]. In this way, the focus has been centred on the integration of functional components within monolithic systems using both lithography and micromoulding technologies. Micropumps are other typical devices used to propel fluids in microchannels. A recent review has been published by P. Woias [10]. A more sophisticated approach has been proposed by M.S. Anderson [11], who presented a combined atomic force microscope (AFM) and Raman spectrometer as a microfluidic device for sampling and trace chemical analysis. On the other hand, the miniaturization of valves for microfluidics [12], or to set up a miniaturized lab-on-valve system [13], constitutes an additional tool for fluid manipulation in microchips. The main examples of integrated processing within microfabricated devices have been directed at linking analytical principles (i.e. detection/CE separation, reactors, designs of microcircuits). As previously mentioned, miniaturization is more than simply the scaling down of well-known systems. The relative importance of forces and processes changes with the scale. Thus, as a consequence of their miniaturized scale, another feature of the analytical

microsystems is the presence of laminar flow (Reynolds number is typically very low), where viscous forces dominate over inertia. This means that turbulence is often unattainable and the transport of molecules is only produced by diffusion, which has direct consequences on the design of this type of microsystem. This constitutes one of the most attractive features, since in most analytical microsystems the diffusion process is very fast, as diffusion effects are inversely proportional to the square of the length. This consequence will be especially relevant during the sample preparation step. However, the ability to efficiently process raw samples (as in classical laboratory tests, directly from the body of a person or an animal, or in field tests), and subsequently to perform the required analytical operations on-chip, will be a key aspect in both the definition of the eventual success and the application of microfluidic systems [14,15].

Whereas the previous points are specific for μTAS approaches, other general requirements can be stated, from an analytical point of view, for a successful miniaturized approach:

(i) The developed analytical microsystems must be close to real-world demands (the objective is to solve analytical problems). Just one of the main challenges of analytical microsystems is dealing with the *real-world interface*. That means understanding the information needs of the client (identification of the problem and its translation to an analytical level), which is a general challenge of any analytical work. But, more important for microsystems, it also means ensuring the representativeness of the results through an appropriate sampling plan and a suitable method of analysis at the particular miniaturized level. This includes achieving the selective and sensitivity requirements through sample treatment and separation/detection.

    In addition, analytical microsystems offer a significant decrease in costs, by dramatically reducing the volume of samples and reagents needed to perform a chemical analysis. This feature also opens up the possibility of processing samples in parallel, which is very useful when the same chemical analysis must be performed many times, as is the case in routine laboratories. This approach is very useful when high-throughput screening is needed. In conventional analyses, handling and processing of the sample is frequently done manually, at least in part, and often in specialized laboratories. However, μTAS allows chemical analyses to be brought close to the place where they need to be performed, independent of both the laboratory and the laboratory personnel. Thus, these integrated analytical systems are very suitable for online measurements.

(ii) The analytical microsystems must perform reliably, in order to be coherent with the present quality assurance requirements in analytical laboratories. In this respect, issues related to the calibration and validation of the methodologies carried out by the analytical microsystems must be taken into account. As the

final objective will be the analysis of real samples, the validation of such a method has to be performed using the samples for which the method is intended.

1.3 Evolution in the Field of Analytical Miniaturization

A. Manz *et al.* established different periods in the evolution of analytical microsystems in a publication in 2002 [16]. Based on this source, and completed with recent developments, Figure 1.4 shows the schematic history of miniaturization in the analytical field. The most representative milestones are briefly described below.

The first period (1975–1989) was characterized by works addressed to the miniaturization of components, such as micropumps, microvalves and chemical sensors, based on silicon technology. The integration of such silicon microcomponents is remarkable for the fabrication of two miniaturized instruments. The first was a miniaturized gas chromatographic analyser [17]. The basic chromatographic device included, in a single silicon wafer, an injection valve and a separation column

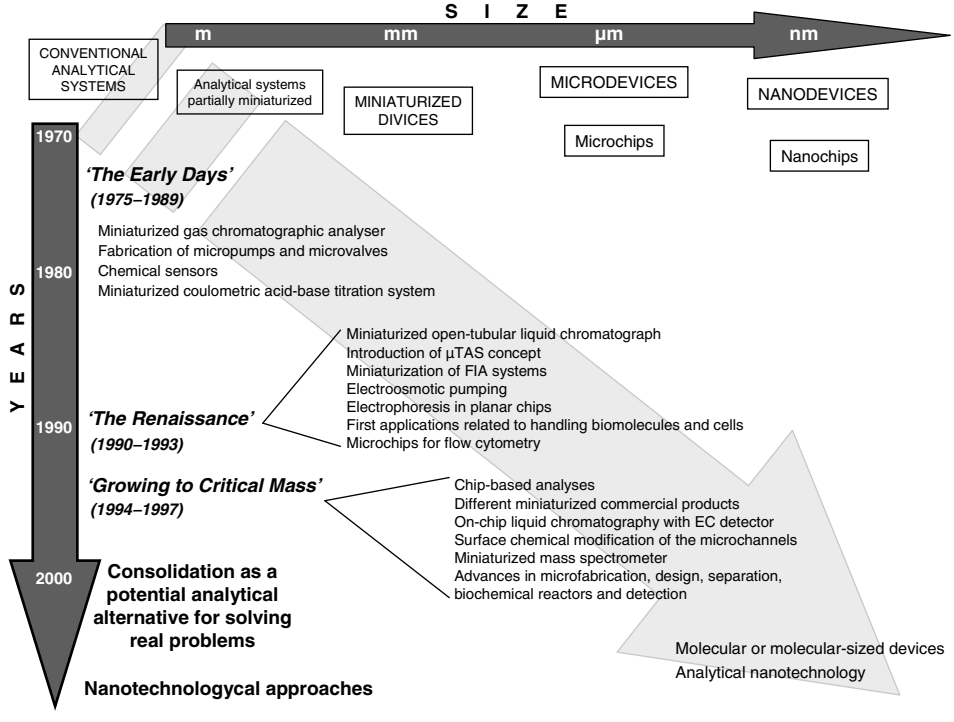

Figure 1.4 *Evolution of miniaturized analytical systems and main milestones (adapted from [16] and completed for years since its publication)*

1.5 m long. A thermal conductivity detector was fabricated on a separate silicon wafer and mechanically clamped on the wafer containing the column. The second type of miniaturized instrument was a coulometric acid–base titration system, employing a solid-state pH-sensitive sensor to determine the acid or base concentration in the sample [18].

The second period (1990–1993) saw the fabrication of silicon-based analysers in 1990, thanks to new developments producing a miniaturized open-tubular liquid chromatograph fabricated on a silicon wafer [19]. This work presented a 5×5 mm silicon chip containing an open-tubular column and a conductometric detector, connected to an off-chip conventional LC pump and valves in order to perform high-pressure liquid chromatography. It was also in 1990 that Manz *et al.* proposed the concept of μTAS [20], in which silicon chip analysers incorporating sample pretreatment, separation and detection played a key role. According to this author, the main reason for this miniaturized approach was to enhance the analytical performance of the existing sensors, due to the poor results in terms of selectivity and lifetime showing at this time. Therefore, the main objective of μTAS, initially, was not the reduction of size, although the advantages of miniaturization were recognized. The miniaturization of a flow-injection analysis (FIA) system, based on stacked modular devices in silicon and plexiglass, was also reported in this period [21,22]. This key device (less than 1 cm^3) conformed to a 3D structure in which more than 10 chips were integrated. Despite the new developments in micropump systems and microvalves for microflow arrangements, the high pressures necessary for transporting in microchannels complicated their practical use. In this way, electro-osmotic pumping was an attractive and feasible tool for the movement of aqueous media in μTAS, particularly when separation was needed. In fact, electro-osmotic pumps are characterized by the absence of mechanically-moving parts and the lack of a specific location of the pump in the manifold. Moreover, the flow in the interconnected channels can be controlled by switching voltages, without the need for valves. These new strategies were critical to the development of electrophoresis in planar chips, used for the first time in 1992 [23,24]. It was also in this period that applications related to the reaction and handling of biomolecules and cells started; for instance, the use of microfabricated chambers to carry out DNA amplification (PCR) or flow citometry.

An important increase in the number of publications related to μTAS took place in the third period (1994–1997). A great variety of chip-based analyses were reported, and different miniaturized commercial products were offered by important instrumentation companies. The modular concept of μTAS was revisited, involving both electrochemical and optical detection, and at the same time, surface chemical modification of the microchannels opened interesting possibilities for miniaturized chromatography. Thus, Cowen and Craston developed an on-chip liquid chromatographer with an electrochemical detector [25]. Another interesting development was carried out by Feustel *et al.* [26], consisting of a miniaturized mass spectrometer

incorporating an integrated plasma chamber for electron generation, an ionization chamber and an array of electrodes acting as the mass separator. Additionally, in this period, significant contributions to microfabrication (covering a broader range of applications and using new materials), separation modes, detection devices and new applications (focussed on biological species, mainly) were made [16].

The last 10 years, since 1998, can be considered a period of consolidation of miniaturized developments, which can be seen as potential analytical alternatives for solving real problems. The wide number and variety of publications is decisive proof of this, although in many cases the developments remain in the research world, with limited implementation in control or routine laboratories. As microfabrication technologies, and particularly the design and production of microfluidic systems, constitute one of the milestones of analytical miniaturization, the important developments in flow-control devices (micropumps and microvalves), interconnections and interfaces, together with bonding techniques and surface modifications, have played a key role in the present analytical miniaturization scene. This is the subject matter of part of Chapter 2 of this book. The application fields of analytical miniaturized devices have been clearly expanded, with a particular impact in the bioanalytical area. Thus, many uses deal with protein and peptide analyses, DNA separation, PCR and performance of inmunoassays or clinical diagnosis. A rise in publications related to cellular applications can also be observed. Selected examples have been reported throughout the various chapters of this book.

1.4 Classification of Miniaturized Analytical Systems and Definition of Terms

Miniaturized systems can be classified according to different criteria. Figure 1.5 presents a classification distinguishing between general criteria and specific criteria applied to analytical work. Thus, from a general point of view, miniaturization is clearly associated with the reduction of size, although the term can be somewhat confusing, and it does not always involve a 'small scale'. It is a relative term, depending on what is understood by the 'normal scale'. This conflict can be resolved by giving to the prefix 'mini-' a broad meaning, representing devices, instruments and systems at dimensions greater than 1 mm (even at the cm range). This is the starting point in Figure 1.6, where the basic classification of the miniaturized systems according to the intrinsic size is represented at two fields: the general and the specific analytical field. From a general point of view, the three basic levels of miniaturization are established by using the prefix mini- (higher than 1 mm, or at the cm scale), micro- (lower than 1 mm) and nano- (lower than 1 μm). Due to the frequent use of these systems with liquid (aqueous) media, the corresponding volumes associated with these levels are included in Figure 1.6, as well as the type of technology involved in the fabrication of the corresponding

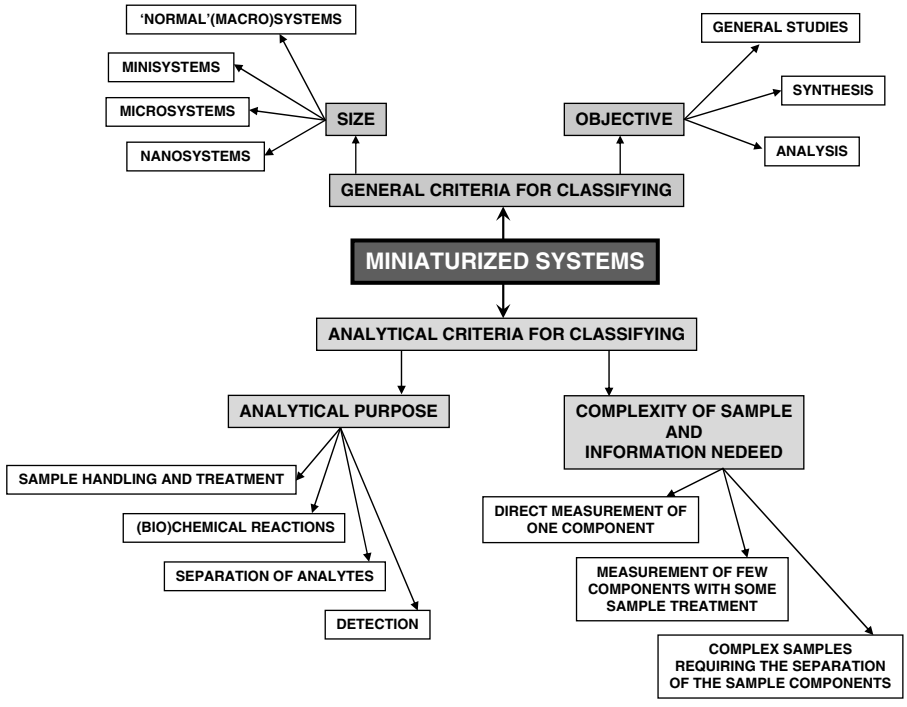

Figure 1.5 *Different criteria used for classifying miniaturized systems*

systems. Thus, conventional technology has commonly produced the wide variety of minisystems existing so far, whereas micro- and nanotechnology were used to produce micro- and nanosystems, respectively. Microsystems are microstructured devices, integrated as structures in the micrometric range, produced by using microfabrication techniques; the term 'nanosystem' refers to a system that has its main structures in the 1–100 nm range. MEMS (microelectromechanical systems) and NEMS (nanoelectromechanical systems) are representative devices often used in micro- and nanoscale works, as well as micro- and nano-fluidic structures.

Analytical miniaturization could be viewed as 'the fact of making to a small scale a part or the whole of the analytical process (see μTAS), or of reducing the size of the different devices involved in the analytical process (see micropumps, microsensors) or the analytical technique itself (see microgravimetry)'. Consequently, when the general-sized concepts are applied to analytical science, the corresponding three levels of miniaturization can be called:

(i) Analytical minisystems or minitechniques, involving some specific devices such as minireactors or minicolumns; or the application of minitechniques, for which commonly the prefix mini- has been replaced by micro- (although not appropriately, according to the general classification). This is the case for

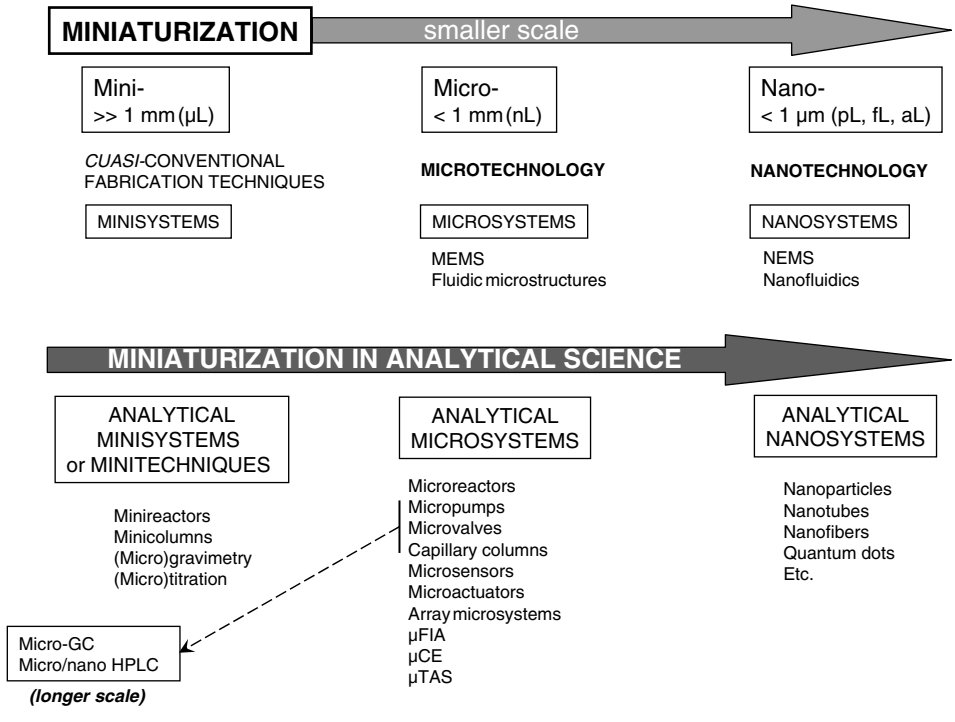

Figure 1.6 *Classification of miniaturized systems based on size criteria*

microgravimetry and microtitration. Other, longer-scale equipment (chromatographic instruments, mainly) has been named micro-GC or micro-/nano-HPLC, not due to the size of the 'miniaturized' equipment, but for the use of minidevices (such as pumps, valves or capillary columns) for handling micro- or nanovolumes, and for introducing separation advantages against normal-sized equipment. From this point of view, the commercially-available capillary electrophoresis (CE) equipment (fabricated at high scale size) could be associated with micro-/nano-CE. Just in this case, the term 'micro-CE' is correctly used when the electrophoretic separation is performed in microchips. This fact is not so frequently used for GC and LC in microchips.

(ii) Analytical microsystems are microstructured devices, integrated as analytical structures in the micrometric range, produced by using microfabrication techniques. They include microdevices (microreactors, micropumps, microvalves, capillary columns, microsensors and microactuators, array microsystems), microtechniques (µFIA, lab-on-a-chip valve, µCE), and the complete analytical process (µTAS).

(iii) Analytical nanosystems are, in fact, systems at nanometer size, built with atomic precision by using nanotechnology facilities. Nanotechnology is a

multidisciplinary field of applied science and technology working at approximately 1–100 nm and dealing with the fabrication of devices at this size. Useful materials, devices and systems at this size range may be obtained by two different approaches. In the 'bottom-up' approach, materials and devices are built from molecular components which assemble themselves chemically by principles of molecular recognition. In the 'top-down' approach, nano-objects are constructed from larger entities without atomic-level control. Examples of analytical nanosystems are nanoelectromechanical systems, nanoelectrodes, etc.

In addition to the proper size classification of the miniaturized systems, from a chemical point of view, miniaturization can be addressed to three main objectives (Figure 1.5): (i) synthesis of compounds; (ii) analysis of samples; and (iii) biochemical studies of cells, microorganisms, etc. In this context, the article by D. Belder presenting the integration of chemical synthesis and analysis on a chip as a potential trend is interesting [27]. This author recognizes that while complex integrated lab-on-a-chip systems have been described for many biochemical applications, similar achievements remain to be made in synthetic chemistry. The main reason for this, besides the often more challenging reaction conditions for synthetic chemistry, is the lack of appropriate detection techniques. Thus, fluorescence detection, which is commonly applied for on-chip detection in bioanalytics, is often not applicable to classical chemical reactions and corresponding products of interest. A promising approach to carrying out chemical reactions and analysis is the use of micro-CE. An example of an integrated reaction/separation device applied to synthetic chemistry and catalyst development has been presented by D. Belder *et al.* [28]. Figure 1.7 shows a scheme of the microfluidic chip, combining a microfluidic reactor with microchip electrophoresis. The chip layout is basically a merged design of the common cross-channel design for on-chip electrophoresis and a typical meandering channel for mixing and reaction. On the other hand, miniaturization technology provides facilities for creating tools with feature sizes matching the dimensions of cells, and enables integration of cell-handling and fluid-manipulation elements [29]. Therefore, microsystems create new opportunities for the spatial and temporal control of cell growth [30]. The additional integration with bioanalytical platforms creates multifunctional microdevices showing great promise for basic biomedical and pharmaceutical research, as well as robust and portable point-of-care devices.

The analytical purpose of the miniaturization can be focussed on sample handling and treatment, the development of (bio)chemical reactions in miniaturized environments, the separation process or the detection. The different incidences of miniaturization in the analytical process steps are discussed in Section 1.7. Microarrays are probably the most representative example of microanalytical systems based on selective and specific (bio)chemical reactions. Their use normally involves

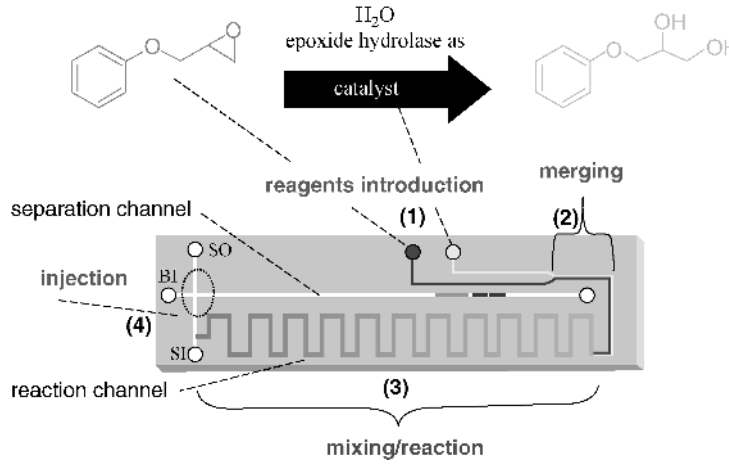

Figure 1.7 *Integrated synthesis/analysis chip (channel width 50 μm) showing the different parts used for introduction of educts through microvials (1), merging zone (2), mixing and reaction microcoil (3), injection cross for the introduction of the reaction products to the microelectrophoretic system (4), and microchannel for the electrophoretic separation of the products (fluorimetric detection). (Reprinted from [28] with permission from Wiley VCH)*

biochemical reagents such as antibodies or genetic material, or specific chemical reagents such as molecular imprinted polymers (MIPs). The second group is constituted by those systems involving (electro)chromatographic separations. Portable GC equipment is a typical example, which can be used to detect volatile compounds in foods. In the case of liquid chromatography, miniaturization results in a high pressure in the system, which makes its use difficult. For this reason, the major tendency is to apply electrochromatographic separations, and hence electrophoretic chips are probably the most developed and studied miniaturized system.

The complexity of samples and the intended information required are two factors taken into account by J.P. Kutter and O. Geschkes [31] for distinguishing between: (i) the direct measurement of one or a few components with no or little sample preparation; (ii) measurement of one or a few components requiring some treatment of sample; and (iii) more complex samples (separation of the components). Undoubtedly, these categories are based on sample complexity and selectivity criteria. Accordingly, different analytical microsystems can be associated with them, for example (bio)sensors (probe-type sensors, flow-through sensors and microsensors in a progressive scale of miniaturization) for the first group. FIA and μFIA are the most appropriate (micro)systems for the second group, whereas μ-LC and μ-CE are ideal for performing microseparations. There is no doubt that information from objects and systems is a relevant part of the requirement for making well-founded and timely decisions [32]. As a response to this high need of information, analytical sciences have developed rapid, low-cost screening methods

that allow the classification of samples as positive or negative [33,34]. Most of the screening methods provide a total index of global response, which permits the classification of samples from the legal, toxicological or quality point of view.

1.5 Theory of Miniaturization

Theoretical considerations can first be established in terms of similarity and proportionality. The *similarity approach* uses dimensionless parameters to consider similarity, whereas the *proportionality approach* uses the characteristic length of known systems versus scaled-down systems to consider proportionality [16]. The former can be used to correlate, in an easy way, experimental results when a great deal of variables are involved, and they are defined in terms of parameters that are assumed to be constant through the whole system. The proportionality approach provides very useful information related to the behaviour of a simple flow system when miniaturized. It assumes the miniaturization as a downscaling process in three dimensions. In fact, as the key factor of miniaturization is the size of the devices, the typical device length, d, can be used as the basis of fundamental changes in the characteristics of miniaturized devices [35]. Thus, Table 1.1 summarizes the basic characteristics of devices at three different sizes: 1 mm, 100 μm and 10 μm. The corresponding volumes associated with these typical lengths clearly decrease, with values of 1 μL, 1 nL, and 1 fL, respectively; as do the number of molecules for a particular concentration of the flowing solution. More important are the three final parameters in Table 1.1. Thus, for instance, as the number of units that can be arranged on a given surface increases with $1/d^2$, for a typical length of 10 μm, 2.5×10^5 devices/cm^2 (250 000 units) can theoretically be integrated per cm^2. The smaller dimensions have a further impact on analytical standard operations such as mixing, separation and detection, because turbulent flow mixing is avoided. Thus, on the microscale, viscous forces dominate over inertial forces, leading to a laminar flow regime. Under this condition, two liquids can co-flow without turbulent

Table 1.1 *Device characteristics for different typical-length d values (extracted from [35])*

Typical length:	1 mm	100 μm	10 μm
Volume	10^{-6} l	10^{-9} l	10^{-12} l
Number of molecules[a]	6×10^{11}	6×10^{8}	6×10^{5}
Diffusion time	15 min	10 s	100 ms
Unit density (devices/cm^2)	25	2500	2.5×10^5
Information density (values/min/cm^2)	1.5	250	2.5×10^6

[a] For a concentration 1 μM.

mixing. Reynolds number characterizes the type of flow, and can be calculated by the following equation:

$$\mathrm{Re} = \frac{\rho r_h v}{\eta}, \tag{1.1}$$

where ρ is the fluid density, r_h is the hydraulic diameter of the capillary, υ is the average fluid velocity and η is the dynamic viscosity. The lower Re, the closer the behaviour of the flow to a laminar flow. In general, systems are considered to be in the laminar flow regime for $\mathrm{Re} < 2000$. This value is even lower than 1 for microfluidic devices. Therefore, diffusion is an important parameter for these microsystems. Thus, as the time that a molecule needs to travel a length d decreases with d^2, for a small molecule (diffusion coefficient $10^{-9}\,\mathrm{m^2/s}$), this results in diffusion times decreasing with the typical length of the device (Table 1.1). For instance, diffusion time is only 100 ms for devices of a typical length of 10 µm. As the diffusion times can be related to the capacity for exchanging molecular information according to the expression:

$$Information = \frac{1}{t_{diffusion}}, \tag{1.2}$$

when the number of volumes is taken into account, an information density parameter can be calculated as a function of the time and surface area, given the values shown in Table 1.1. These values theoretically increase with the fourth power of d. This is a critical aspect, because it is the basis of the high-throughput capability which characterizes miniaturized analytical systems.

The influence of relevant parameters on the behaviour of a miniaturized system can be evaluated from proportionality considerations. For this purpose, the parameter of interest must be defined as a function of space (d) and time (t), which are the key variables of the miniaturization. This allows knowledge of the characteristics of a parameter after downscaling, without having knowledge of other constants associated with the material. Under this model, miniaturization is viewed as a simple 3D downscaling process based on d factor. Depending on whether or not the other key variable, time, is constant, two different situations can be distinguished:

(i) *Time-constant system*, for which diffusion is of lesser importance. In this case, the timescale is the same for the miniaturized system as for the large system. Variables characterizing the time parameters, such as transport time, response time and analysis time, remain the same. This is the situation characterizing simple transportation systems and FIA, where an analyte is injected into a flowing carrier stream for subsequent analysis. According to A. Manz *et al.* [36], when a time-constant system is scaled down, the linear flow rate decreases by a factor d, volume flow rate by d^3 and voltage by d^2 (Table 1.2). Hence, the main advantage is the reduced consumption of sample, carrier and reagent solutions.

Table 1.2 *Scaling factors for miniaturized analytical systems, where d represents a typical length in the system (extracted from [36])*

Parameter	Time-constant system	Diffusion-controlled system
Space	d	d
Time	constant	d^2
Linear velocity	d	$1/d$
Volume flow rate	d^3	d
Pressure drop[a]	constant	$1/d^2$
Voltage[b]	d^2	constant
Plate number	—	contant

[a] Laminar flow conditions; [b] For electro-osmotic pumping.

(ii) *Diffusion-controlled system*, in which the time is regarded as a surface and proportional to d^2. Parameters such as molecular diffusion, heat diffusion and flow characteristics are important because they control the separation efficiency. Therefore, in these cases, timescale needs to be considered. As the diffusion time on the travel length is proportional to d^2, a 10-fold downscaling produces a 100-fold reduction of related time parameters (for instance, the analysis time). On the other hand, as Table 1.2 shows, the pressure drop scale is proportional to $1/d^2$, meaning that for a 10-fold downscaled system, a 100 times higher pressure drop is required to generate the same flow. If an electrical potential is applied to produce an electro-osmotic flow (EOF), the generated flow remains the same when the electric field is kept constant. The separation efficiency (expressed through the plate numbers) is also unaffected by miniaturization, but is directly proportional to the applied voltage. Obviously, the applied voltage is limited by the microsystem heating (Joule effect), although for miniaturized systems, lower currents are used, and the higher surface-to-volume ratio allows a good heat dissipation. Based on this fact, ultrafast, high-efficiency separations can be achieved in miniaturized diffusion-controlled systems.

1.6 Features of Miniaturized Analytical Systems

The previous section pointed out some of the features and advantages of miniaturized systems. Now, Figure 1.8 summarizes some of the main characteristics of analytical microsystems, establishing their relationship with the quality of the generated information. Automation (human participation reduction) and simplification are two features commonly associated with miniaturization. The other characteristics listed in Figure 1.8 show clear influences on the speed of result generation (high throughput), the amount of information (simultaneous or multiparametric)

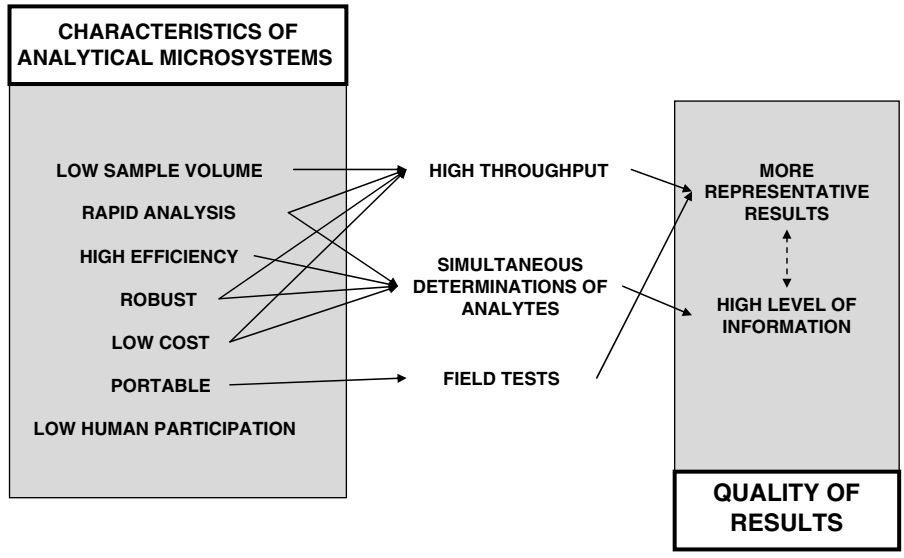

Figure 1.8 *Main characteristics of microsystems and their effects on the quality of the analytical results provided*

and the autonomy given for microsystems allowing field tests (portability). These characteristics represent very advantageous aspects for the corresponding analytical methods using miniaturized systems. The development of such microsystems requires the miniaturization of the electronic and mechanical parts of the system. The microtechnology used in the miniaturized environments could also be understood as integrating the possibilities offered by microfabrication and microfluidics. The term 'microfluidic' is used to denote any process that involves the use of small amounts of fluids, such as 10^{-9}–10^{-18} litres, in channels with a small diameter (tens to hundreds of micrometres). Hence, μTAS are examples of microfluidic systems. From an analytical point of view, these microsystems offer a number of useful capabilities: (i) the ability to use small quantities of samples and reagents; (ii) the possibility of carrying out separations with a high resolution; (iii) the use of low-cost setups; and (iv) the short time taken to perform complete analyses. In addition, microfluidics also exploits characteristics of fluids in microchannels, such as laminar flow. This allows new capabilities in the control of the molecules in space and time.

One of the most important characteristics of microfluids is the dramatic difference between the physical properties of fluids moving in large channels and those travelling through micrometer channels, mainly the turbulence or its absence (laminar flow). On large scales, fluids mix convectively, as inertia is often more important than viscosity. However, in microsystems, the fluids do not mix convectively and, consequently, two fluidic streams that merge in a microchannel will

flow in parallel, without any turbulence. Therefore, the diffusion of molecules across the interface is the unique phenomenon responsible for mixing. When effective mixing is required, it is necessary to introduce specific devices to accomplish this. Another important characteristic is the presence of electro-osmotic flow, which, as in CE, is a consequence of the ionization or surface charge of the microchannels. Therefore, when an electrical potential is applied across the microchannel, the fluid moves as a plug, with a characteristic planar flow profile.

1.7 Incidences of Miniaturization in the Analytical Process

(Bio)chemical analysis is performed through the so-called analytical process, which integrates a group of steps and substeps connecting the sample with the corresponding results. Miniaturization can affect a single step/substep, various integrated steps/substeps or the entire process. Figure 1.9 illustrates these possibilities, taking the analytical process as the central part, and considering it the 'analytical black box', which can be the basic subject of integrated analytical (micro)systems; or the specific sequence of the three main steps: preliminary operations, signal measurement and transduction, and data acquisition and processing. Hence, the upper part of the figure represents a (micro)system performing the whole process, whereas the lower part includes the different analytical standard operations involved in the process. This view allows a description of the incidence of miniaturization in the analytical process as: (i) the partial miniaturization of step(s), devices or equipment;

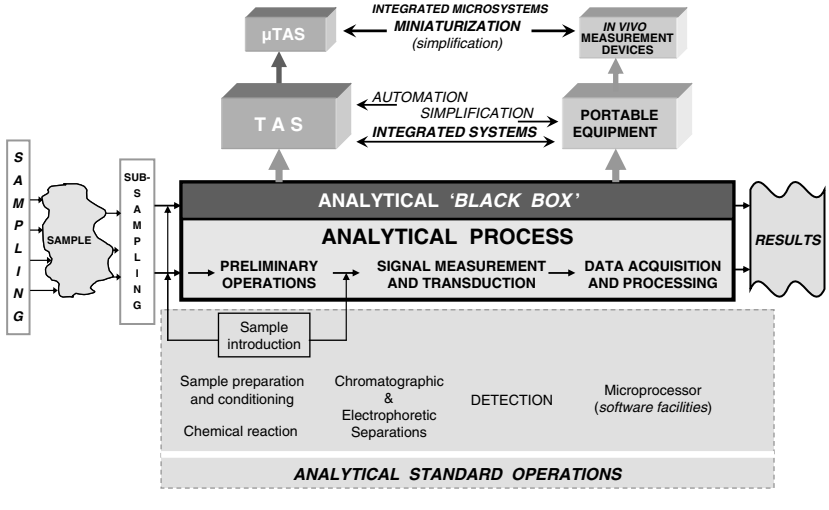

Figure 1.9 *Incidence of miniaturization in the whole analytical process or individually in the different analytical standard operations involved in the process*

or (ii) the miniaturization of the integrated systems performing the entire analytical process, in which miniaturization should be viewed in combination with automation and simplification of the process.

1.7.1 Miniaturization of the Steps of the Analytical Process

The main approaches used for the miniaturization of the steps making up the analytical process are given below, pointing out the present challenges and evaluating the main strengths/advantages and weaknesses/disadvantages existing in each. Taking into account the natural development of the miniaturization devices in analytical works, the logical sequential steps of the analytical process have been inverted, in order to present them in accordance with the increasing difficulty of their miniaturization.

Detection Techniques

Detection has been one of the main challenges for analytical microsystems, since very sensitive techniques are needed as a consequence of the ultra-small sample volumes used in micron-sized environments. In principle, a wide variety of detection alternatives can be used in microfluidic systems [37]. Laser-induced fluorescence (LIF) was the original detection technique and is the most used detection scheme in CE microchips, due to its inherent sensitivity [38,39], given by the ease of its focusing. This characteristic, together with the fact that molecules of biochemical relevance are fluorescent in many cases, is an important reason for the wide use of LIF in microfluidics. Today, LIF has attained a position as a standard detection technique for microchip separation [39]. However, the high cost and the large size of the instrumental setup of LIF are sometimes incompatible with the concept of μTAS. Also, tedious derivatization schemes are needed to use LIF with nonfluorescent compounds.

The most important alternative to LIF detection is, without any doubt, electrochemical detection (ED). ED is very important because of its inherent miniaturization without loss of performance and its high compatibility with microfabrication techniques. Likewise, it has a high sensitivity, its responses are not dependent on the optical path length or sample turbidity, and it has few power-supply requirements, which are all additional advantages. As proof of the prominent role of ED, see the recent publication of excellent reviews [40–43] and others papers [44–48].

The main challenge in CE–ED coupling has been the conflict between the high voltages used in the electrophoretic separation and the potential used for the detection. However, at microscale this drawback is over. Three strategies have been employed: *end-channel*, *in-channel* and *off-channel* electrochemical detections. In end-channel detection the electrode is placed just outside of the separation channel, which involves the alignment of the electrode. Separation voltage has a

minimal influence on the applied potential in the electrochemical detector because most of the voltage is dropped across the channel. For in-channel detection, the electrode is placed directly in the separation channel using an isolated potentiostat. Off-channel detection involves grounding the separation voltage before it reaches to the detector by means of a decoupler. Electrode placement in off-channel detection is similar to that in in-channel detection, but the separation voltage is isolated from the amperometric current through the use of a decoupler. Conceptually speaking, the decoupler effectively shunts the separation voltage to ground and a field-free region is created where analytes are pushed past the electrode by the EOF generated before the decoupler. Since no decoupler is necessary, end-channel configurations offer the following advantages: they are simple and rugged, electrode replacement is feasible, and microfabrication facilities are not strictly required. However, the main drawbacks are the alignment of the electrode with the outlet of the channel, and the loss of separation efficiency due to the distance between the end of the channel and the working electrode. This separation distance is also crucial for the signal-to-noise ratio obtained and can lead to a complete loss of the analytical current. In both in- and off-channel configurations, the analytes migrate over the electrode while they are still confined to the channel, thus eliminating the band broadening often observed with end-channel alignments. However, in these configurations, microfabrication facilities are usually needed and in addition the nature of on-chip miniaturized electrodes limits the ability to modify and periodically clean the electrode surface.

Conductimetric detection on microfabricated devices has recently been developed. It constitutes an important possibility for detection in analytical microsystems. In comparison to amperometry, conductimetric detection is less sensible, but it is a universal detection technique and has been applied as a detection mode in CE microchips, both in the galvanic (a pair of electrodes is placed in the separation channel for liquid impedance measurement) [49,50] and in the contactless (no contact between the pair of electrodes and separation channel solution) [51–54] mode. Contactless conductimetric detection is preferred for three main reasons: (i) the electronic circuit is decoupled from the high voltage applied for separation (no direct DC coupling between the electronics and the liquid in the channel); (ii) the formation of glass bubbles at the metal electrodes is avoided; and (iii) electrochemical modification or degradation of the electrode surface is prevented. Both contacts and contacless detectors integrated into a microchannel require physical connection to read out electronics placed inside or even outside the microdevice. Conductimetric designs are often very sophisticated and microfabrication facilities are required; however, simple and easy alternatives have also been proposed. These alternatives involve the deposition of conducting electrodes on the cover plate of microchips only, avoiding the clean-room laboratory infrastructure and showing significant advantages over the other approaches due to their simplicity [51].

As already mentioned, the inherent strength of ED versus other detection modes is its compatibility with miniaturization (without loss of performance), plus advantages in microtecnology requirements (microfabrication facilities). It also constitutes, itself, an important improvement on LIF implementation, in which only one setup is possible. The possibilities in the implementation of ED can be understood as follows (see Figure 1.10): (i) analytical configurations showing complete integration of the electrochemical cell in the separation system (hard lithography); (ii) a partial integration, in which the separation system is microfabricated in one layer using soft lithography and the electrochemical detection is first deposited in another one; and (iii) the partial integration of the electrochemical cell (reference and auxiliary electrodes) with the separation system, allowing a replacement of the working electrode.

In addition to LIF and ED, there are other detection approaches for analytical microsystems, but, from a realistic point of view, they are less developed than those discussed above. All of them are the focus of a recent and very interesting investigation. For instance, integrated UV and visible light absorption detectors have been tried on microchips [55].

Recently, the use of *thermal lens microscopy* (TLM) in combination with microchips has demonstrated an interesting analytical potential, as T. Kitamori *et al.* have clearly reported [56]. TLM is one of the most powerful absorption microspectrometries, and its sensitivity is comparable to LIF. TLM can be applied to both fluorescent and nonfluorescent analytes, thus expanding its versatility. Typical applications of microchip TLM include extraction, immunoassay, flow injection,

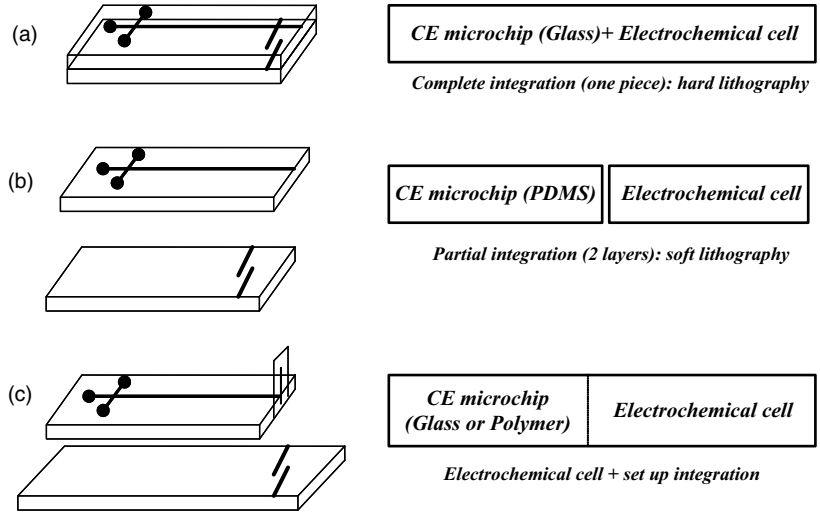

Figure 1.10 *Micromachining of electrochemical detectors integrated in microchips. (Reprinted from [101] with permission from Elsevier)*

enzymatic assay, *in vitro* bioanalysis and CE methods [56]. Complicated (bio) chemical processes can also be carried out by microchip TLM, as has recently been demonstrated [57]. Although the evolution of TLM instruments has been impressive, the nonminiaturized environment, the sophistication and the cost of such instruments are the main disadvantages of TLM microsystems.

Relevant challenges in coupled detection techniques are focused on the interfaces, particularly on making the micro flows from microsystems compatible with the flow requirements of detection units. Mass spectrometry (MS) and ICP are examples of approaches in this area. MS, as one of the most powerful detection and identification techniques, has been successfully interfaced with microdevices, showing significant possibilities in the corresponding microchips from a practical point of view [55]. Some attempts have also been made to devise an interface between a chip and ESI (electrospray ionization) [58]. Some reports described an efficient sample introduction into the mass spectrometer. However, its size and price remained a challenge to its widespread use. A very interesting new interface, microchip capillary electrophoresis with inductively coupled plasma spectrometry for metal speciation, has been also reported [59,60], opening the possibility of new applications involving inorganic analytes. Detection in miniaturized analytical systems is systematically covered in Chapter 6.

Separation Techniques

The potential benefits of miniaturization were quickly applied to separation techniques. As M. Szumski and B. Buszewski reported, miniaturized separation systems are currently divided into 'column' and 'chip' systems [61]. The former are related to the miniaturization of column chromatographic systems, while in the latter the separation is performed in the channel of a chip device. The first group is characterized by the presence of micro- and nano-HPLC [62], whereas the second is more related to μTAS. As we mentioned before, diffusion effects are very fast in micron-sized environments. These effects have a direct advantage in separation techniques, since the reduction of the size of microchannels accelerates sample-stationary phase equilibria. In general, miniaturized separation techniques such as chromatography, electrophoresis and electrochromatography have great advantages over techniques at conventional scale. The decrease of the scale provides very rapid separations, versatile channel designs, very small sample volumes and low reagent consumption. The increase of the molecular interaction also makes the chemical process highly efficient [55,63–65].

CE was one of the earliest examples of μTAS, and constitutes one of the most representative examples of an analytical microsystem. Using CE microchips, analysis times can be reduced to seconds and extremely high separation efficiencies can be achieved. Over the past decade, the most active field (as judged by publication outputs) of analytical microsystems development has been the transference from

conventional separation techniques (macro scale) to planar chip formats. Therefore, without any doubt, CE microchips have a prominent role in the miniaturization field. In fact, they can be considered a synergic combination of miniaturized CE and microchip technological developments. On one hand, this combination involves those features derived from the analytical performance itself, such as the ability to simultaneously assay hundreds of samples in a matter of minutes (or less); rapid analysis combined with massively parallel analysis arrays, which should yield ultrahigh throughput; and the low volume of sample needed (at picolitre level), potentially prepared onboard for complete integration of sample preparation and analysis function (i.e., derivatization). On the other hand, the synergic combination conceptually involves those features derived from important aspects involved in their miniaturization. That means the easy microfabrication of a network of channels using materials of well-known chemistry, exhibiting by themselves a good electro-osomotic flow, and the possibility of using the electrokinetic phenomena to move fluid – namely valveless microdevices – and subsequently increasing their analytical possibilities. Since electrokinetics is easily applied (just a pair of electrodes is needed), EOF-driven flow has been successfully implemented using different types of material in the manufacturing channel, with glass the most commonly used. Microfabrication on polymers is faster and cheaper than that on glass, so these materials have great possibilities for mass production. In contrast, glass chip presents the best EOF, and the chemical modification on the surfaces of its microchannels is better understood than that in polymers. These features involve larger versatility in chemical analysis. The two major polymers used on chips, PDMS and PMMA, both present good optical transparency and low EOF. However, if PDMS is oxidized in a plasma discharge it presents similar EOF to glass material. Furthermore, as PDMS is an elastomer, the process of bonding substrate to the cover plate is easier, whether reversible or irreversible bonding (by oxidation in a plasma discharge) is chosen. Nowadays, PDMS is overtaking PMMA in microchip use. Another important advantage it has is the possibility of obtaining disposable CE microchips [66]. In addition, micro-CE has shown distinct advantages when compared to conventional CE, such as reduced analysis times and extremely high separation efficiencies obtained. Indeed, it has been theoretically indicated that analysis times can be reduced through a reduction in channel length or an increase in separation voltage [65].

Many of the benefits mentioned for CE microchips could equally be applied to downsized chromatographic techniques; however, the literature covers relatively few examples of chip-based chromatographic instruments compared with chip-based CE devices, even though the first analytical instrumentation on-chip was a gas chromatograph [67] and later a liquid chromatograph [68]. This is unsurprising as CE is almost perfectly suited to miniaturization, while the miniaturization of chromatographic systems involves some technical challenges, such as the microfabrication of valves and pumps, which are generally not faced in CE. On the other

hand, the early on-chip liquid chromatography (LC) examples showed the potential advantages that miniaturization could provide [68]. These advantages included superior efficiency compared to conventional LC, facile positing of detection cells and low unit cost. In addition, the use of an opened tubular system instead of a normal packed column was deemed advantageous due to the shorter analysis times and low pressure drops for a given performance. The common conventional-scale alternatives of packed and tubular columns in LC have also been transferred to the microscale. Although packed columns may be desirable, the introduction of stationary phase material into microfabricated channels is not a trivial process. Frits must be fabricated within the channel structure to retain the packing and a high-pressure interface between the chip and an external pump must be applied. In addition, due to the reduced channel, the packing process is difficult to carry out and can often lead to nonuniform density particles at channel walls, reducing separation efficiencies. Consequently, the majority of initial on-chip chromatographic methods employed a tubular approach [65]. The difficulties associated with packing microfabricated channels can be eliminated if the packed bed is replaced by a porous bed formed by *in situ* polymerization from organic monomers. The process of bed formation is easy, since a low-viscosity monomer solution can be introduced by vacuum or pressure into the microfluidic channel prior to initiation [69]. In this way, it is important to note that electrically-driven chromatographic separations are especially attractive within chip-based systems, due to a lack of pressure gradients and reduced fabrication complexity. Due to the fact that the mobile phase runs by electro-osmosis, the flat flow profile significantly reduces band-broadening when compared to conventional LC methods. Chapter 4 presents the miniaturized systems for analyte separation based on hydrodynamic flow, while the microsystems based on electro-osmotic flow are described in Chapter 5.

Since, as stated before, one of the primary advantages of micromachining analytical instrumentation is the ability to facilitate processes or fabricate structures that are extremely difficult or even impossible to recreate on the microscale, future research is focussed in this area. The concept, which can be thought as *in situ* micromachining, involves the creation of micron-sized particle support structures on the surface of a planar wafer [70,71]. This line of research is now underway, and is opening exciting new analytical possibilities.

Sample Preparation

One of the first approaches of miniaturization in sample treatment can be identified with the use of microdevices/units for performing nonchromatographic/electrophoretic separation techniques as sample treatment tools [72]. Solvent microextraction (liquid phase micro-extraction, LPME) and solid phase microextraction (SPME) procedures are two examples.

As stated earlier, most advances have been made in separation and detection schemes. However, many of the necessary analytical steps (such as sampling and sample pretreatment) are still performed outside the chip. An important effort has been made to move them on-chip in the last few years. Thus, some excellent reviews on this topic have been published [73,74]. Although this analytical process step is less developed than the separation and detection steps, potentially it has a very exciting future. The combination of two important aspects underlines this statement: the possibility of designing complex layouts in connection with the presence of fast diffusion (statistic transport), and the simplicity of applying electrokinetics (directed transport). Table 1.3 lists the main strengths and weaknesses in the integration of sample preparation in analytical microsystems, and has been taken as the basis of the discussion of this section. Although microfabrication and microfluidics are very well adapted to many sample preparation steps, several challenges – such as miniaturization of components, direct analysis of raw samples, sampling and sample introduction – are still present. However, the good current knowledge of microfluidics and the possibility of creating sophisticated layouts can be drawn as the main technological strengths. In addition, derivatization schemes in different formats, using the well-implanted detection modes (LIF and ED), have already been successfully introduced [7,41,64].

Sample treatment Filtration, extraction/preconcentration (including clean-up in some cases) and derivatization are the most common analytical operations that can be carried out in sample preparation. Prior sample filtration is, probably, the most essential step for analyses using microfluidic systems. Raw samples and other already-treated samples (in aqueous media) require basic filtration before analysis. Due to the small dimensions of typical microstructures, particulates can cause serious operational problems, providing sites for blockage. The simplest solution is

Table 1.3 *Strengths and weaknesses in the integration of the sample preparation in analytical microsystems*

(A) MAIN TECHNOLOGICAL SUPPORTS
Microfabrication (layouts)
Microfluidics (fast diffusion, electrokinetics)
(B) MAIN STRENGTHS
Derivatization schemes successfully introduced (pre- and post- strategies/LIF and electrochemical)
(C) MAIN WEAKNESSES (challenges)
Miniaturization of some components
Direct analysis of real samples
Representativeness of the portion of sample analysed
Sampling/sample introduction in the microsystem
Changes of samples within a set of analysis

to filter reagents and samples prior to their introduction. Therefore, it is desirable to integrate a sample filtration system on-chip prior to the analysis. Two approaches have been employed: structurally-based filtration (controlled by the manufacturing process) and diffusion-based filtration (controlled with diffusion). Structurally-based or microfabricated filters have been proposed in popular architectures such as frits, pillar structures and flow restrictions within fluidic channels to mimic conventional filters [75]. H-filter structures have been most used in diffusion-based filtration processes [76]. Filtration can be induced by allowing the analytes of interest to migrate across a laminar boundary (between a sample and a solvent stream) while unwanted heavier particulates are retained in the original fluid stream. It is very important to underline that both approaches are principally linked to miniaturization itself: the first because of the well-known technology integrating multiple micropieces into a single microdevice; and the second because it works on the basis of an inherent property in microfluidics: presence of laminar flow. However, the limit of the structurally-based filters is the resolution limit of the manufacturing process, while the great advantage of the diffusion-based filters is that the whole process is controlled by physics, although a very sophisticated technology is also required.

Liquid–liquid extraction could play a prominent role in miniaturized systems. The high surface-to-volume ratios and the short diffusion distances typical within microfluidic environments, combined with laminar flow conditions, offer the possibility of performing liquid–liquid extraction within microchannels without the need for agitation. The main challenge is to induce appropriate electro-osmotic flows when common organic solvents are used. The few examples found in the literature have also employed H-filter strategies [76].

Two approaches to performing solid phase extraction in microfluidics have been proposed: first, coating channel walls with a high-affinity stationary phase, and second, packing the microchannels with the stationary phase material [66,72,73]. The advantages and disadvantages are clearly defined in miniaturized environments. Coating-channel approaches depend on the available surface area for interaction, and in micron-sized channels the contact surface is very small. A simple way to increase the surface area is to pack the microchannels with stationary phase; however, the packing process is not easy, and this route is often avoided. A very attractive possibility, compatible with miniaturized dimensions, is to replace conventional stationary phase materials with a continuous porous bed *in situ* formed from polymerization of organic monomers. The process of bed formation is easy, since a low-viscosity monomer solution can be introduced by vacuum or pressure into the microfluidic channel prior to initiation [66,75]. An excellent paper showing the power of integration filtration, concentration and separation was recently published [77]. In this paper, filtration, concentration and separation are integrated on to microchip. Filtration consists of an array of seven thin channels (1 μm deep) which come together into one channel (5 μm deep). The input of the thin channels is

communicated with sample reservoir and the sample loading is electrokinetically carried out. Sample concentration is performed by solid phase extraction. The separation channel is coated with C18 (1.5–4 μm) particles. Then, simply by increasing injection times, the analytes are retained and concentrated in the separation channel, and afterwards eluted with the appropriate solvent.

In some cases, these basic operations for sample treatment have been seen as micro unit operations (MUO) [78], and used for developing microintegrated chemical systems on the basis of a concept similar to electronics: a chemical process can be constructed like an electronic circuit, but in place of the resistor, capacitor and diode, mixing, extraction, phase separation and other operations are integrated.

Field-amplified sample stacking is a common method for sample preconcentration in electrophoretic systems. However, its implementation into a chip platform is not easy. The initial difficulty associated with sample stacking in a microfluidic format is the control of the analyte zone during the stacking and separation procedures. This often requires the use of relatively complex channel networks and voltage programmes to stack the analyte zone [73,74].

Derivatization on microchip is well established in analytical microsystems. The reason for this development can be found in the traditional use of LIF as a sensitive detection system, since the earliest times. Again, the role of microfabrication in the design of complex microcircuits offers a unique route in sample pretreatment and especially in derivatization schemes, where the process is carried out before or after analyte separation and before analyte detection. In other words, the high degree of functional integration (reagent mixing, product separation and post-column labelling) provides an elegant indication of the potential benefits of microfluidic systems. Derivatization schemes carried out prior to the separation (precolumn) or immediately prior to the detection (post-column), have been proposed using LIF and ED detections. Thus, derivatization in fluorescence has been well implemented into microchip in connecting with LIF detection [79]. A group of works uses a very attractive strategy based on the combination of suitable pre- and post-channel layouts with bioreagents such as enzymes and antibodies, along with electrochemical detection [80,81]. It is important to remark that, in some cases, these are truly lab-on-a-chip devices [82,83]. In fact, the generation of electroactive products using selective bioreagents such as specific enzymes, class-enzymes and antibodies in adequate pre- and post-column derivatization schemes has revealed the potential of these strategies in the simplification of treatments of complex samples. This line can be used for developing other sophisticated layouts for sample treatment when real samples are analysed in these microsystems.

Sampling and sample introduction Sample introduction and the representativeness of the small size of the sample introduced are two important aspects of microfluidic systems. Most chip-based systems still adopt a discrete and often

manual approach to on-chip sample loading and sample changing. Such practice seriously lowers the overall throughput and counteracts the advantage of achieving fast separations. On-chip loading and changing of a series of samples has become a limiting factor in real-world applications. Efficient assays of real-world samples will require the incorporation of a continuous sampling capability (from the external environment) or rapid sampling of multiple discrete samples. Such an ability to continuously introduce real samples into micro-sized environments should make analytical microsystems compatible with real-life applications. Different devices, based on pressure-driven [84–86], hydrodynamic [87] and autonomous polymer-loading [88] techniques have been reported for sample injection.

Finally, one of the most important aspects to consider is the continuous introduction of samples, which requires an interfacing system between the macroenvironment (μL–mL) and the microchip (nL–pL) in order to achieve an efficient sample change with low carryover. Hence, a suitable design of interfaces, similar to the hyphenated detection techniques, is necessary. Although bibliography is poor in papers dealing with this subject, various approaches have been proposed in excellent works. In one, using a microfluidic matrix device, sample introduction was performed through a wide-bored microchannel, drilling an access hole through the sample reservoir [89]. Very recently, the use of a sharp sample-inlet tip was reported [90]. An excellent report dealing with trends relating to different configurations of sample introduction interfacings was recently written by Fang, showing a critical vision of different strategies used by his research group [91]. This report shows that the trend is to separate the sample-introduction channel (SIC) with hydrodynamic flow from the separation channel with electrokinetic flow, thus avoiding totally pressure-driven flows. This was achieved by introducing a sample-loading channel (SLC) between the SIC and the separation channel. The SIC acts as a flow-through sampling reservoir and the sample is electrokinetically loaded into the SLC from the SIC. The SLC connects with the separation channel. This split-flow strategy is improved by a flow-through sampling reservoir featuring a guided overflow design. This array avoids Poiseuille flows, maintaining equal liquid levels for the sample, the buffer and the waste reservoirs. A fast and simple sample introduction was recently reported [88]. It consists of the use of a sharp sample-inlet tip alternately placed in the sample and the buffer vials. This tip was fabricated by sharpening the inlet side of the chip with a diamond saw. A good reproducibility was obtained (100 repetitive flow injection measurements resulted in a response with an RSD of 3.7%). C.-W. Huang and G.-B. Lee have proposed an interesting contribution consisting of micro-autosamplers for discrete sample injection and dispensation in microchips [92]. It is a promising approach toward realizing the μTAS concept. All these aspects related to the miniaturization of sample treatment are reported in more depth in Chapter 3.

1.7.2 Integrated (Micro)systems for the Performance of the Entire Analytical Process

As Figure 1.11 shows, an ideal analytical measurement system should be characterized by:

(i) Portability and self-operation, in order to avoid or reduce the sampling step and allow the possibility of performing field tests.
(ii) Either the noninvasive measurement approach or the performance of in-line measurements. These features allow the minimum perturbation of the sample.
(iii) Facilities for the maintenance of the system and large lifetimes. Alternatively, reusability at low price.
(iv) Self-calibration incorporated in the system.
(v) The possibility for the incorporation of quality-control activities, in order to assure a reliable response.

Sensors were one of the first approaches to performing the entire analytical process. They represent by themselves a miniaturization and simplification of the analytical process, although the initial expectation of a wide field of application was demonstrated to be unfounded by limitations in selectivity and lifetime. But in any case, sensors constitute a growing area of interest with a strong social and industrial impact. In fact, sensors provide fast response with reduced costs. Ideally, a sensor is a device that provides an analytical signal for a specific compound present in a raw sample in a direct, reversible, continuous and reliable manner. From a practical point of view, most of the sensors reported in the literature fall short of the requirements of the above definition. Hence, a more realistic definition of a sensor is that it is a sensitive microzone where a (bio)chemical reaction occurs that is connected to or integrated in a physical (optical, electrochemical, thermal, mass-sensitive) transducer [93]. Through this connection to an instrument, analytical information can be produced *in situ* in real time. The problem of interferences is partially solved by the so-called 'biosensors', which are defined by IUPAC as self-contained integrated devices that are capable of providing specific quantitative or semiquantitative analytical information using a biological recognition element (biochemical receptor) in direct contact with a transducer element. Biosensors including transducers based on integrated circuit microchips are known as 'biochips'. The biological recognition element used in a biosensor can be an enzyme, an antibody/antigen, a nucleic acid, a cellular structure or a biomimetic receptor.

The term 'microsensor' describes a sensor whose active sensing area is in the micro range, while the sensing device itself is much larger for easier handing. 'Microelectrode' and 'ultramicroelectrode' are terms used in electrochemistry used instead of microsensor, although electrodes with smaller radii (nanometric size) are currently used [94]. The small size of these electrodes makes diffusional mass

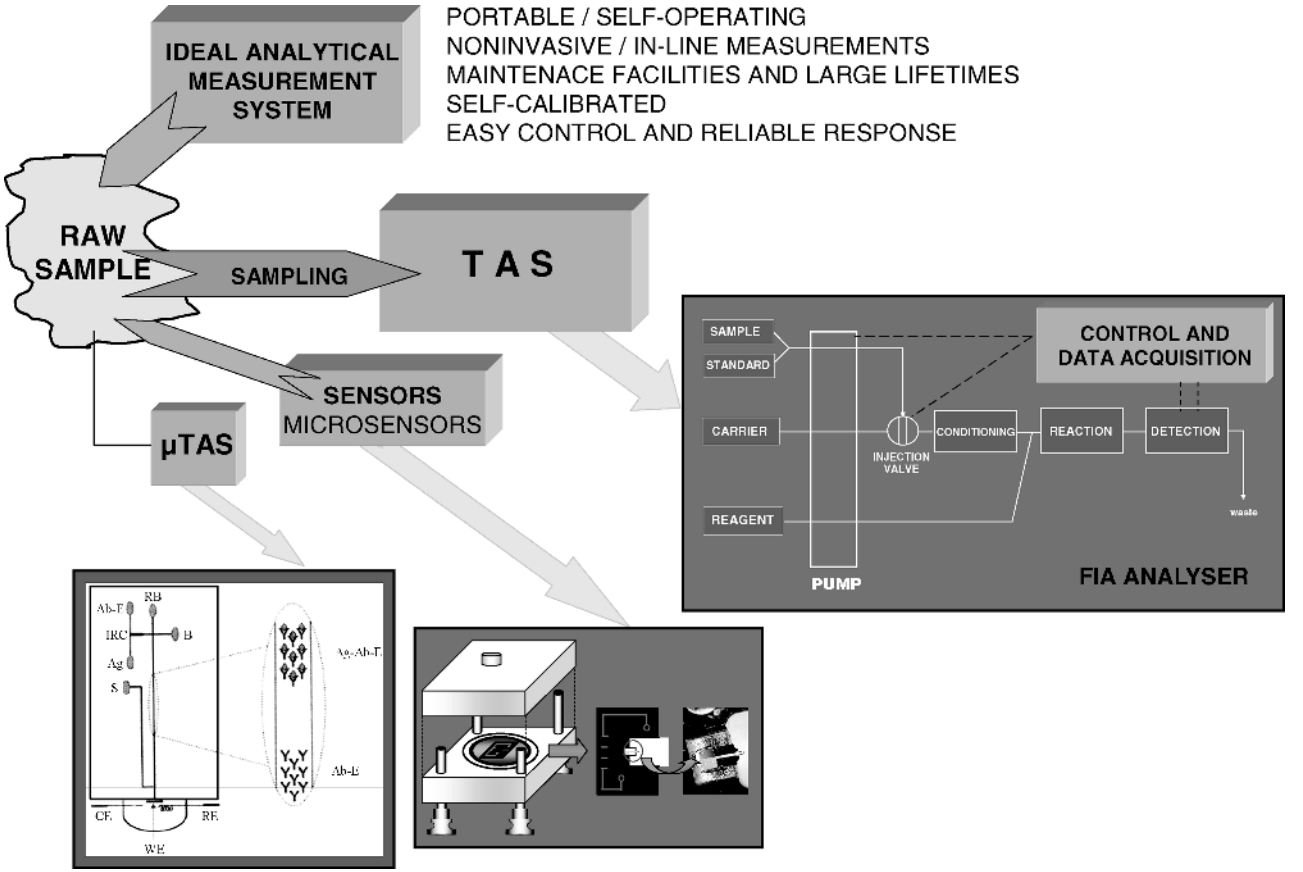

Figure 1.11 *Different approaches to the implementation of the entire analytical process, combining various degrees of automation, simplification and miniaturization (see text for details)*

transport extremely efficient and double-layer capacitance very small, attaining greater signal-to-noise ratios and lower detection limits than those of macroelectrodes [95,96]. Chapter 7 reports the miniaturization of the entire analytical process through micro(nano)-sensors.

The TAS approach is addressed to meet these ideal features, integrating the different analytical standard operations of the analytical process. Portable instruments can be considered as a particular case of TAS, designed to operate outside the laboratory (miniaturized portable analytical systems are reported in Chapter 9). Both are integrated analytical systems, with a high level of automation and simplification for the intended analyses. Miniaturization commonly exists in these systems, but in a relative sense, with respect to other laboratory alternatives using conventional equipment to carry out the same application. On the other hand, the following step giving integrated microsystems (μTAS or *in vivo* measurement devices) is strongly characterized by miniaturization, although simplification and automation are implicit in these microsystems. Chapter 8 is devoted to the miniaturization of the entire analytical process through μTAS approaches.

As illustrative examples, Figure 1.12 shows four common categories of integrated systems miniaturizing the entire analytical process. The first one (Figure 1.12a)

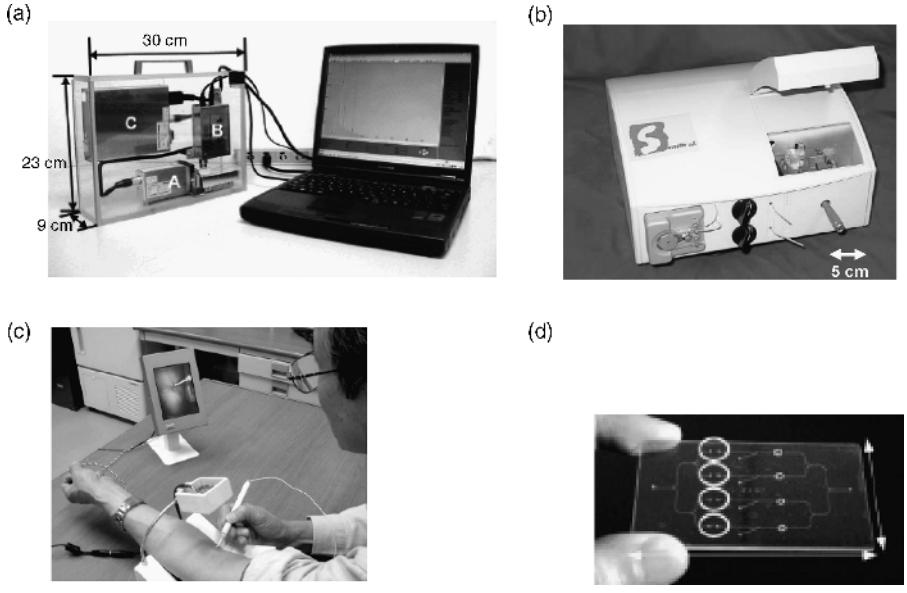

Figure 1.12 *Examples of different levels of miniaturized analytical systems: (a) portable X-ray fluorescence equipment (Reprinted from [97] with permission of American Chemical Society, Copyright 2007); (b) small analyser for SPR with optical biosensor detection (Reprinted from [98] with permission from Elsevier); (c) portable miniaturized equipment for health care (Reproduced from [99] with permission of IEEE, Copyright 2007); (d) μTAS for micro-ELISA analysis (Reproduced from [78] with permission of IEEE, Copyright 2007)*

is a portable total reflection X-ray fluorescence spectrometer. Despite the macro-size (cm level), it represents a clear miniaturization with respect to a conventional X-ray fluorescence spectrometer operating at laboratory-routine work [97]. The second category (Figure 1.12b) is represented by small-sized laboratory equipment used for the determination of environmental organic pollutants based on a surface plasmon resonance (SPR) optical biosensor device [98]. The third (Figure 1.12c) is a type of portable miniaturized equipment for health care used for home medical diagnosis. It corresponds to a calorimetric three-item measurement chip (triglyceride, total-cholesterol and HDL) combined with an electronic blood-collection system (6 μL of whole blood) [99]. The final category (Figure 1.12d) is represented by μTAS, as the multichannel micro-ELISA chip in the image shows [78].

From a practical point of view, the reliability of the information generated by these integrated microsystems is a key factor. Performance tests for the proper microsystem and the appropriate validation of the whole analytical method must be carefully carried out. Within these activities, calibration must be planned in tandem with the miniaturized equipment work, especially in the case of portable systems. In this context, the integration of the calibration with the particular manifold or scheme of the microsystem should be taken into account. Major difficulties can be found in miniature analysis instruments for measuring trace levels of gases, commonly used for field tests. In these cases, standard gas *in situ* generation for onsite calibration is a very useful alternative. Thus, recently S.-I. Ohira *et al.* described one of these systems for on-site checking or onsite calibration of a micro gas analysis system, μGAS [100]. The key part of this arrangement is shown in Figure 1.13. The source reagent (R1 in Figure 1.13) was mixed with the desorbing solution (R2) and reacted in the mixing coil (MC), then introduced into a micro-channel gas generator. Generated vapour permeated through the membrane and was extracted into the air stream flowing on the opposite side of the membrane. This microsystem was used to generate H_2S, SO_2, CH_3SH and NH_3.

1.8 Outlook

Analytical microsystems constitute an important trend within analytical chemistry science. The high number of scientific contributions published in this field so far is a good reason to imagine that one of the next objectives will be dealing with application issues and expanding their use to laboratories at bench level. Miniaturization presents different difficulties and problems regarding the different steps involved in the analytical process [101]. Detection schemes, such as LIF and ED (amperometry), are well established in analytical microsystems; nevertheless, novel designs, especially in ED, are being developed. Although MS was studied from earliest times because it is a very powerful technique [102,103], its use in μTAS is nonetheless not widely proposed due to its size and price. Recently, F. Foret and

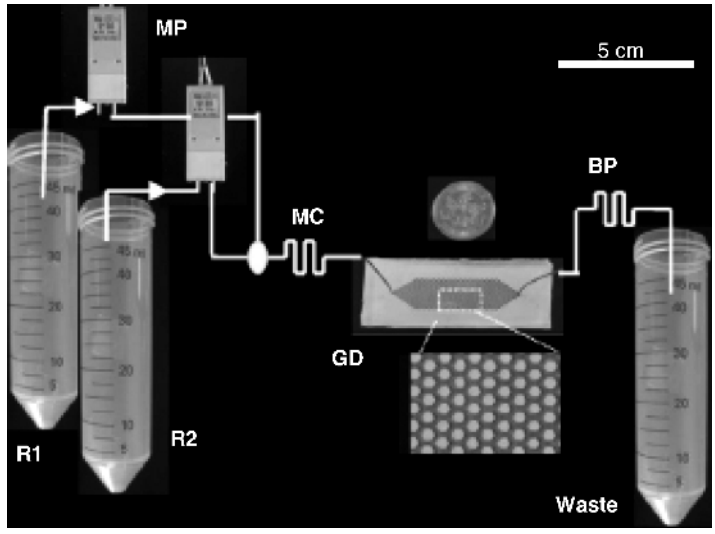

Figure 1.13 *Liquid flow system for gas generation with microchannel gas desorber. R1: source reagent; R2: desorbing solution; MP: liquid minipums; MC: mixing coil; GD: gas desorber; BP: back pressure tube. (Reprinted from [100] with permission from Elsevier)*

P. Kusý published a review on microfluidics for multiplex MS analysis [104]. As the authors recognize, the development of microfluidics–MS coupling is still in its infancy, despite the analytical potential of these microsystems. They pointed out that 'the future success will depend on the willingness of both the instrument manufacturers and the users to adopt this technology in practice'. This will require a clear demonstration of the technology's superiority and robustness. Regarding the detection capabilities, LIF and amperometry are not very suitable for small molecules, including inorganic species. Alternative detection schemes, such as conductivity, ICP-AEE or ICP-MS, are necessary and consequently research on new detection systems for analytical microsystems is still open. Additionally, the use of TLM for detection and imaging studies performed in microchips will open interesting new possibilities.

Separation on chips is primary developed using CE, which constitutes the most representative example of μTAS. In microseparation, new tools such as the design of new analysis schemes and strategies including the use of nanoparticles, employment of specially designed polymers and new cover materials will be developed.

Sample preparation at microscale level has been less developed than both detection and separation techniques. In general, some of the most relevant promises of lab-on-a-chip devices, such as integration of all laboratory functions in order to get practical sample treatments, commercialization, truly handheld operation and ease of use, are coming, but today are not a reality. However, the possibilities of using electrokinetics as directed fluid transport, of creating suitable layouts for

problematic needs, and of laminar flow, allow us to think of future developments as a near reality.

Total integration [105] and, especially, world-to-chip interfacing are considered the major challenges, particularly in high-throughput applications requiring frequent sample change, such as online continuous process monitoring. More specifically, various world-to-chip interfacing schemes are being developed to meet different requirements of sample and microfluidic chips, in terms of sampling rate, sample introduction, sample consumption, precision, stability and degree of automation. Two clear trends are the development of more integrated, rugged, portable and fully automated sample introduction systems, and systems based on various hydrodynamic principles outside of the electrokinetic environment.

On the other hand, a basic trend with respect to analytical applications is the exploration of the possibilities of these microsystems in other important analytical areas, ranging from medical diagnosis and pharmaceutical screening to food and environmental control [106]. This expanded use can only be fulfilled by versatile and highly sophisticated analytical devices. One exciting trend in miniaturization is the use of micro/nanodevices for biological and bioanalytical studies of cells, genes, proteins and individual molecules. Different alternatives are available for cell trapping in microfluidic chips [107], demonstrating that miniaturization technology provides facilities for creating tools with feature sizes matching the dimensions of cells, and enables integration of cell-handling and fluid-manipulation elements and the chemical analysis of single cells [108]. Also, microsystems create new opportunities for the spatial and temporal control of cell growth. Further, as recently reported by J. El-Ali *et al.* [109], typical unit operations such as cell growth, treatment, selection, lysis, separation and analysis have been demonstrated to be implemented using μTAS. But, as they pointed out, robust approaches to fabrication, integration and packaging (such as communication with the macroenvironment) remain major areas of research. Gene analysis on a single chip is now a possibility, although with some challenges [110]. Microfabricated electrophoresis devices offer several advantages over conventional methods for rapid genotyping [111] and DNA sequencing [112]. Proteomic analysis based on microfluidics is also the subject of interesting developments. *Electrophoresis* devoted a special issue to this topic in 2006 [113], and other interesting contributions have appeared since. In an exciting article, H. Craighead discussed future lab-on-a-chip technologies for interrogating individual molecules [114]. As he reported, these advances allow the manipulation and measurement of individual molecules, and '*the adaptation of these approaches to lab-on-a-chip formats is providing a new class of research tools for the investigation of biochemistry and life processes*'.

It can be said that the first generation of lab-on-a-chip devices is starting to work, and in the future, successive (more sophisticated) generations will introduce themselves as a new reality in analytical laboratories. In this new generation, a further integration of sample collection and sample preparation, improving

lab-on-a-chip for world connections in order to reach the goal of 'total integration', will be necessary. New approaches to avoid the major shortcomings of chip-based analytical systems, such as the risk of the analyte adsorption on walls and at interfaces (which is especially high in low-volume analytical systems), and the optical interference at the walls of chips in the detection point, will help to solve practical problems. The concept of *digital microfluidics* in the late 1990s can be addressed in this way, as it involved the manipulation of discrete volumes of liquids on a surface by mechanisms such as electowetting, thermocapillary transport and surface acoustic wave transport [115]. Sample levitation can open the exciting new approach of *lab-on-a-drop*, representing an alternative for preparing nano-pico sample volumes without contamination from solid walls or other external objects, or between samples [116].

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