



Review

Miniaturization through lab-on-a-chip: Utopia or reality for routine laboratories? A review

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HIGHLIGHTS

- ▶ LOC possibilities on routine laboratories are reviewed.
- ▶ LOC selected contributions have demonstrated the practical usefulness.
- ▶ LOC features – routine laboratory requirements are discussed.
- ▶ Main LOC ‘niche’ for practical developments is pointed out.

GRAPHICAL ABSTRACT



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ABSTRACT

Micro total analysis (μ TAS), also called “lab-on-a-chip (LOC)” technology, promises solutions for high throughput and highly specific analysis for chemistry, biology and medicine, while consuming only tiny amounts of samples, reactants and space. This article reports selected contributions of LOC, which represent clear practical approaches for routine work, or presenting potentiality to be transferred to routine analytical laboratories. Taking into account the present LOC state-of-the-art, we identify various reasons for its scarce implementation in routine analytical laboratories despite its high analytical potential, as well as the probably main “niche” for successfully practical developments is suggested.

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1. Introduction

For the past two decades, the demand for continuous and fast-response measurements using small volumes and low concentration samples has been the driving force for research in many areas of analytical chemistry [1–4]. Two solutions have been proposed in order to satisfy these needs: the first is based on the use of selective chemical sensors [5] or micro-electromechanical sensors [6], and the second on the so-called total analysis system (TAS) approach [1]. Although sensors have the advantages of in situ analysis and fast response, the main limitations are the high sensitivity and selectivity required for the detection of tiny amounts of analytes in complex sample mixtures. Ideally, a TAS performs all the component stages of a complete analysis in an integrated and automated fashion. These stages can include sampling, sample pre-treatment, chemical reactions, analytical separations, analyte detection, product isolation and data analysis. The philosophy of TAS has enabled enhancements in on-line chemical analyses, however, significant drawbacks still exist. These include slow sample transport, high reagent consumption, poor separation efficiencies and the need to fabricate interfaces between distinct components.

The main idea behind TAS was to offer a valuable alternative to the existing (bio)chemical sensors rather than to reduce the size of analytical devices [7]. In 1979, the first silicon-based miniaturized gas chromatograph [8,9] was presented as a new microfluidic integrated system [10], initiating a real "microfluidics revolution", and the significant development of miniaturized analytical systems [11,12]. The device reported by Manz et al. consisted of an on-chip open-tubular column (1.5 nL volume) and a conductometric detector with a 1.2 pL volume detection cell. At the same time, Manz et al. [13] proposed the micro total analysis system (μ TAS) concept, opening a new era in the field of analytical chemistry. The main aim of μ TAS was to fabricate integrated systems ideally able to perform a number of functions in an automated way, in a miniaturized system [14–18]. μ TAS has grown exponentially and evolved into a more general area of research encompassing different types

of microfabricated chemical, biochemical and biological systems also popularly referred to as lab-on-chip (LOC). The objective of these miniaturized systems, or microfluidic analysis systems, is to automate standard laboratory processes and to conduct chemical and biochemical analysis in a miniaturized format. The speed and cost-efficiency, combined with small reagent consumption and less waste generation are obvious advantages. Moreover, the results of research can be obtained within a few seconds, instead of hours or days.

After the introduction of the first LOC commercial product based on collaboration between Caliper Technologies Corp. and Agilent Technologies [19], the field of LOC has continued to gain maturity. The importance of LOC is reflected in both the growing number and the improved quality of articles published on this topic. LOC receives great input from a spectrum of scientific and engineering disciplines and equally touches on an incredibly diverse number of analytical chemistry applications, such as clinical diagnostics [20–23], nucleic acids [24–29], proteins [30–35], cell and tissue studies [36–40], environmental monitoring [41–44] and general research [45–49]. In this sense, LOC is truly interdisciplinary in nature and has served as a focal point to bring together the different research fields. To date, there exists a set of excellent review articles [50–53] that study LOC devices from different stand points and cover a subset of their components.

In fact, the first microfluidic technology was developed in the early 1950s when efforts to dispense small amounts of liquids in the nanolitre and picolitre range were made, providing the basis for today's ink-jet technology [54]. In general, the predicted or newly discovered striking behaviors of fluids at micrometer scale, the constantly emerging new analytical devices with increasing complexity and capabilities of physically or chemically harnessing microfluids in finely controlled manners, the appealing future applications of these devices in chemistry, biology, physics and material sciences [55–57] have spurred another world-wide "gold rush" among scientists from various scientific disciplines.

The aim of this article is to discuss the way to match lab-on-a-chip features with routine laboratories needs. Since the introduction of the LOC concept [13] to the latest achievements [51], a large number of contributions have been published, in some cases with potential applicability and transferability to routine laboratories. The discussion should be focussed on practical aspects such as the development and the analytical validation of robust, rapid, low cost and safety procedures and methodologies, producing reliable information. In fact, for routine laboratories, results produced in analytical reports must be representative, traceable and with acceptable uncertainty. From an analytical point of view, four main steps must be followed to ensure practical interest in lab-on-a-chip. First, research and development work has allowed the fabrication of the particular platforms, which must be assessed for their intended uses (second step). Transferability of this R&D work is related to the appropriate analytical validation in order to provide robust and standard operational procedures for

practical use (third step). Analytical validation ultimately involves the reliable response and its capability for use in routine analytical work, including the corresponding internal quality control activities (fourth step). If we consider this sequence of steps, the present state-of-the-art clearly shows the prevalence of the first two steps, whereas steps 3 and 4 require additional further works.

2. Main milestones AND features in miniaturized analytical systems

Reyes et al. [55] established different periods in the evolution of analytical microsystems in a publication in 2002. Based on this source, and completed with some recent developments, Fig. 1 shows the history of miniaturized analytical systems in the periods 1975–2011. The figure includes some selected references corresponding to the main achievements.

Analytical miniaturization could be viewed as “the fact of making to a small scale a part or the whole of analytical process, or reducing the size of the different devices involved in analytical process or the analytical technique itself” [11]. Automation (human participation reduction) and simplification are two features commonly associated to miniaturization. Other characteristics present clear influences in the speed for generating the results (high throughput), the amount of information (simultaneous or multi-parametric), 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 needs the miniaturization of the electronics 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. From an analytical point of view, these microsystems offer a number of useful capabilities: (i) ability to use small quantities of samples and reagents; (ii) possibility to carry out separations with a high resolution; (iii) use of low cost set-ups; and (iv) short time for performing the complete analyses. Microtechnology must be applied to each of the steps of the analytical process (sample introduction and preparation, separation, and detection) in a miniaturized environment. Both, microfabrication (micromachining) and microfluidics, are inversely related to the degree of complexity in the analytical chemical process. Indeed, while microfabrication plays a preeminent role in separation and detection systems, physical control of the process has a prominent role in all steps of sample preparation. In addition, the microfabrication required in sample preparation is very sophisticated. Fig. 2 shows a simplified scheme of the main steps from planning to the final analytical use of a specific microsystem. This figure also summarizes the main points of: (i) design of the microsystem according to some previous technical specifications (connected to the analytical problem to be solved); (ii) simulation and optimization by use of an appropriate computerized software; (iii) the proper fabrication as a previous prototype using clean-room, microfluidic and microelectronic facilities for micromachining and final integration by packaging; and (iv) technical

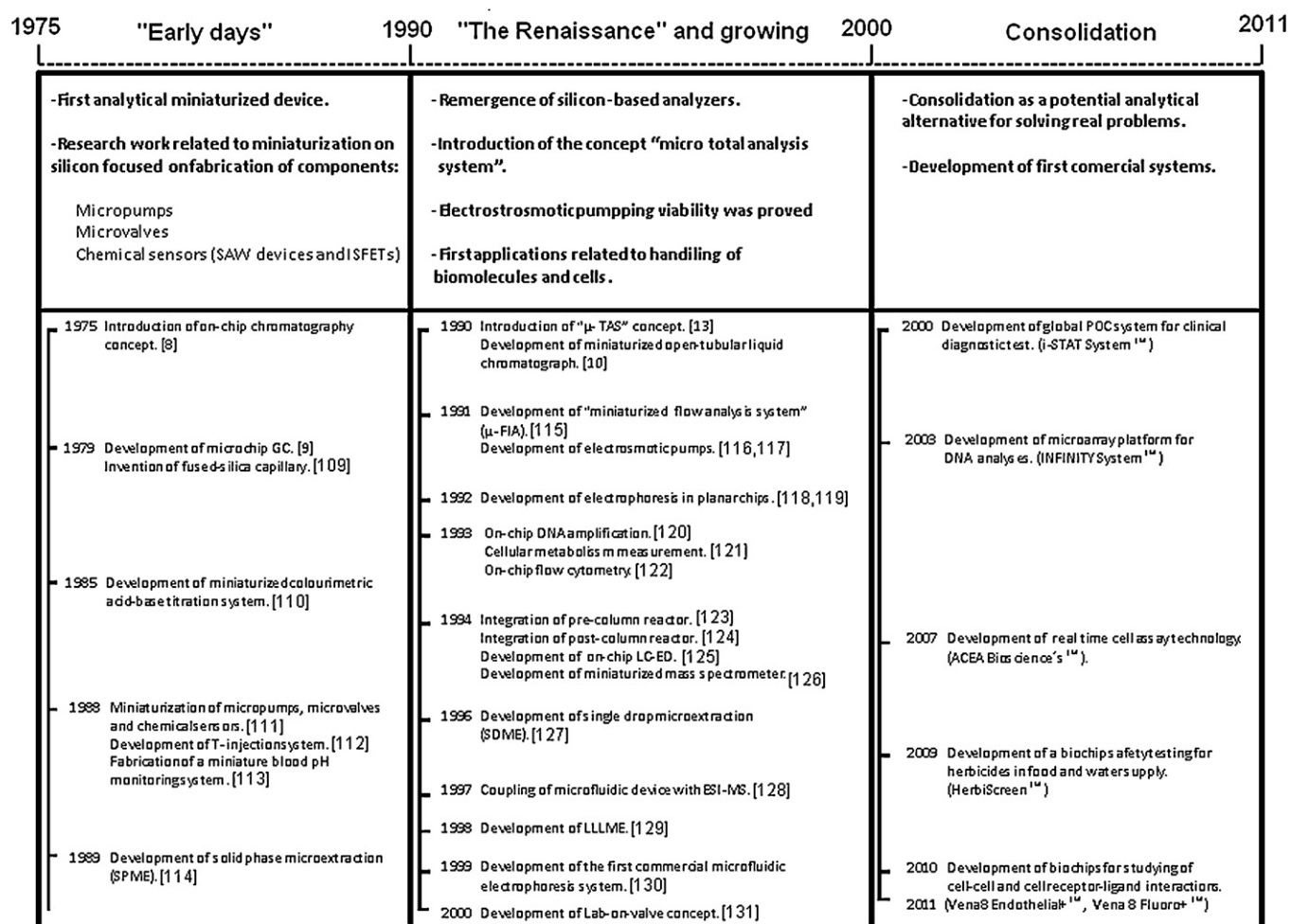


Fig. 1. Evolution and main milestones of μTAS (see Refs. [109–131]).

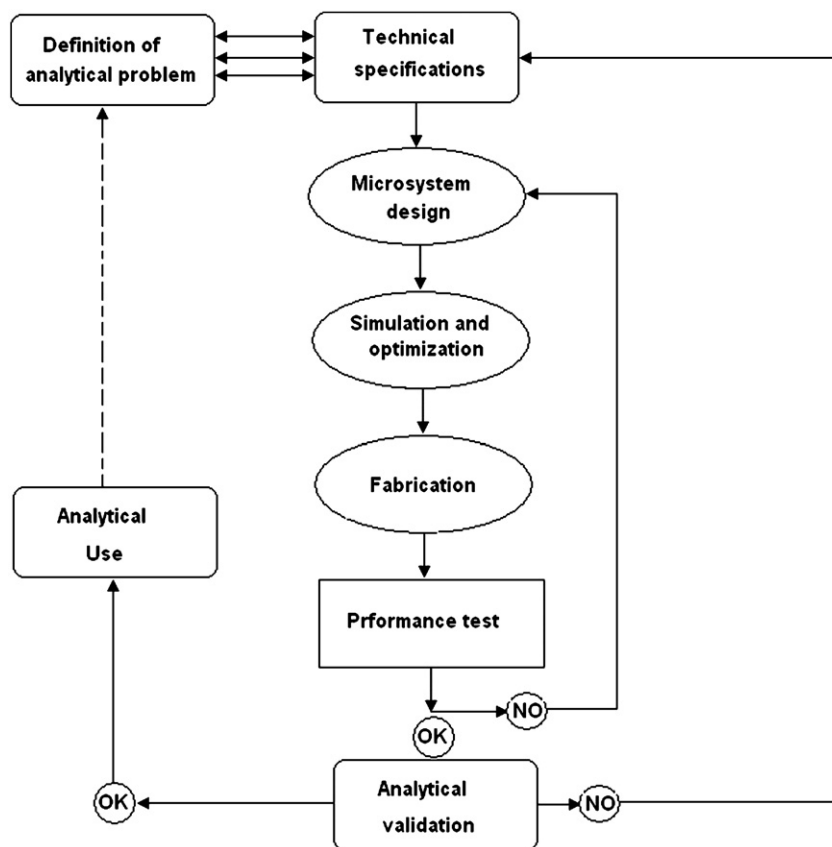


Fig. 2. Simplified scheme of the main steps to be followed in the development analytical microsystem.

verification of the prototype, and if it passes, analytical validation for its intended use. If the expected technical and analytical purposes are achieved, massive fabrication can be planned if needed.

Becker [58] describes a good example process chain for two of the main manufacturing processes for microfluidic devices: (i) using glass as a substrate material (structuring either with wet chemical etching, sandblasting [59] or photostructuring [60]), and (ii) using polymers as substrates using replication methods like injection molding or hot embossing [61]. This author divided the process into four distinct phases. The first phase contains the design phase of the device as well as the production of the design-specific tools for the later manufacturing, i.e. a photomask in the case of glass etching or the replication master (mold insert) in the case of injection molding. In this phase, it is absolutely vital to consider aspects of manufacturability. During this so-called “design-to-manufacturability” phase [62], issues like the following have to be taken into account:

- Reduce the number of parts by combining functions.
- Reduce the number of microstructured parts by reducing fluidic levels as much as possible.
- Use a robust design, i.e. avoid vulnerable structure details if possible.
- Minimize assembly steps.
- Design so it is only possible to assemble the components in an unambiguous way.
- Use popular standard/preferred sizes.
- Eliminate or simplify alignments/adjustments.
- Do not specify tolerances tighter than necessary.
- Do not specify surface roughness smoother than necessary.

After designing the microfluidic structure and the generation of the mask/master, the next phase is the etching of a glass wafer or

the molding of a polymer part. At this point, one has a microstructured substrate. In order to make a functioning microfluidic device out of this microstructured substrate, it is necessary to perform the third phase called back-end processes. Typical back-end processes are the drilling of fluidic access holes, the dicing of individual parts out of a wafer, the bonding of a cover lid or another fluidic layer onto the microchannels, the surface treatment for the binding of specific molecules or the suppression of unspecific binding and the tuning of the hydrophilicity or hydrophobicity of the device. Finally, in industrial production, quality control measures are an indispensable part of the production, constituting phase 4 of the overall production process.

The miniaturization of capillary electrophoresis (CE microchips approach) has opened interesting possibilities for transference to routine work. This technology is undoubtedly the most rapidly advancing and fruitful branch to date in miniaturized separation systems. The reason for this dominance lies partially in the inherent simplicity of its fabrication and operation, combined with its unique features with respect to separation speed, sample injection and reagent consumption. CE microchips employ channels etched into a planar substrate, which are based upon microfabrication techniques developed in the semiconductor industry. Early applications of CE microchips employed glass or quartz for chip substrates because they are optically transparent and exhibit EOF properties similar to those of fused silica. A number of other materials, including polymers/plastics, have since been investigated [11]. Photolithography and micromolding are used to fabricate microchannels for sample injection, CE separation and analyte detection. Once all solutions, including those of the sample, are loaded, the samples are typically transferred electrokinetically into an injector region. Their components are then separated by application of high voltage, and afterwards detected with a suitable detection system. Moreover, CE microchips have the potential to

simultaneously assay hundreds of samples in a matter of minutes or less. The rapid analysis combined with massively parallel analysis arrays should yield an ultrahigh throughput. These microchips typically consume only picolitres of sample and may potentially be prepared onboard for a complete integration of sample preparation, separation and detection. Also, a couple of key advantages of the use of these planar devices/substrates are their ease of manufacture and the fact that microfabrication techniques can be adapted for mass production. These features make CE microchips an attractive technology for the next generation of CE instruments. A wide variety of applications involving DNA and protein analysis, and small molecules in various fields such as clinical, food and environmental can be developed. In this way, Hopwood et al. developed a fully integrated instrument for the analysis of multiplex short tandem repeat DNA profiles from reference samples [63]. This instrument is loaded with a DNA processing cartridge which incorporates on-board pumps and valves which direct the delivery of sample and reagents to various reaction chambers to allow DNA purification, amplification of the DNA by PCR, and collection of the amplified product for delivery to an integral CE microchip. Rech et al. reported a compact four color microchip electrophoresis-based genotyping system for DNA sequencing [64].

Rhazi et al. used a commercial microfluidic LabChip®90 protein assay system (Caliper Life Sciences, Hopkinton, MA), for qualitative and quantitative analyses of polymeric proteins, such as high-molecular-weight glutenin subunits (HMW-GS) [65]. The authors found that the CE microchip analysis provides comparable resolution and sensitivity to conventional RP-HPLC for identification of the HMW-GS, but at a time scale of approximately 100 times faster (45 s per sample analysis versus 80 min for RP-HPLC). The results show that the high throughput CE microchip method can be used for routine identification and quantitation of glutenin subunits, in particular for screening wheat quality and wheat cultivar development activities, where large numbers of samples must be evaluated. On the other hand, He et al. recently reported a tuneable thick polymer coatings for on-chip electrophoretic protein and peptide separation [66]. The coating was readily prepared in short time with little complication using the confinement effect and the photolytic selection of the coated region, thus generating surfaces with different properties in different regions, allowing considerable control of the device behavior and performance.

In clinical analysis, CE microchip is the most common technique used in miniaturized analytical devices, due to its special suitability for miniaturization [11]. Obubuafo and co-workers have reported a PMMA microchip for the analysis of thrombin in blood plasma using affinity gel CE of aptamer protein complexes [20]. A plug-based microfluidic chip capable of performing agglutination assays for ABO and D (Rh) blood typing and blood group has been developed by Kline et al. [67]. Nie and Fung reported a microchip-CE for frontal analysis of free bilirubin at clinically significant levels [23].

Avila et al. developed a useful application for a rapid sample screening of vanilla flavors [68], which can be used for confirmation of common frauds in this types of food samples [3]. Wang et al. developed a method for determining thiol-containing degradation products of V-type nerve agents in water samples [69]. The separation and detection of three of these compounds were achieved in only 4 min in glass CE microchip with amperometric detection. Llopis et al. used a magnetic bead-based assay for the detection of carbofuran (a carbamate pesticide toxin) on a glass microchip [70].

3. Micro total analysis systems and portability

In essence, a μ TAS is a device that improves the performance of an analysis by virtue of its reduce size. However, some consequences derived from this initial objective, as low consumption

of sample and reagent or miniaturization, have become more and more important to for expanding this concept. There are two interesting concepts, related with μ TAS and instrumentation, in which definitions would be interesting to clarify. These concepts are “handheld” and “portability”. We believe that the two share almost the same constraints and tradeoffs as concerns size and performance; moreover, the border between them can be blurry at times. In fact, the former is a portable device, whereas the latter is not necessarily handheld. By definition, a handheld device can be held in one's hand, indicating a high degree of compactness, and a portable one has been designed to be light and small enough to be easily carried or moved [71]. Both have the important characteristic of being able to take them to the sample and not vice versa. This is also important for occupational safety monitoring (so-called direct-reading instruments) and analyzing potentially hazardous samples such as carcinogens, highly toxic chemicals, and potentially explosive atmospheres where real-time data and remote monitoring is advisable or mandatory to enable rapid decision-making [72].

Because portable instruments are likely to be used in different, at times remote locations, the available power is usually limited. There are two parallel ways to deal with this constraint: the first is by designing the instruments for reduced energy consumption, and the second by using more efficient batteries.

In this way, great efforts have been made in the development of ultra-low-power-electronic and optoelectronic components, including control inputs and built-in capabilities for stand-by or sleep mode operations, when required, in most processing electronics units such as operational and instrumentation amplifiers (OA, IA), microcontrollers (MCU), digital signal processors (DSP), and microprocessors (MPU). This leads to negligible energy consumption by the processing electronics compared with the light source consumption [71]. On the other hand, the reason of this growing interest in portability is because this fact is much more than an easier way of transport. Portability involves the possibility of carry out in situ analysis, on very different fields and levels. Hence, the development of this characteristic will be different depending on the field of application. The most important fields of application for portable systems are clinical-biochemical, environmental and food analysis. Capitán-Vallvey and Palma have reviewed the recent developments in portable and handheld analytical instrumentation over the last decade (2000–2011) [71]. In clinical-biochemical field portability has lead to the decentralization of clinical analysis, being able to carry out by the patient itself in a simple and regular way. This fact has an especial importance for these people which need very frequent analysis for a specific clinical parameter, as for example diabetic people. Hand-held glucose meter, used all over the world, is the most representative example of this type of devices. Its existence has changed the life of millions of persons. Nowadays, the ambition of scientist is going on and research is focused in the development of devices which are able to carry out a separation and determination of key metabolites, related with a specific pathology, or drugs of human fluids as saliva, blood or urine. Babichenko et al. have patented a portable, reliable, automated and simple device for fast and accurate illegal drug detection [73]. The system is based on spectral fluorescence signature technology (SFS), also known as synchronous spectrofluorimetry, a technique that shows advantages in the analysis of mixtures of fluorescent compounds in aqueous solution when their spectra show strong overlapping. The excitation–emission–intensity matrix generated for each sample is processed by an expert system in a computer. The system was tested for cocaine and marijuana in the presence of common adulterants and diluents. Lee et al. have reported a lab-on-a-chip system with a disposable polymer device capable of detecting glucose lactate and partial oxygen from 3.5 μ L in 100 s [74]. Detection of antibodies to HIV in saliva has been reported using a timer actuated cassette by the Bau group [75]. In this portable system,

the automated timely pumping of various reagents was driven by a spring-loaded timer. A portable disk-based enzyme-linked immunosorbent assay (ELISA) system is reported by Lee et al. [76] to test infectious diseases from whole blood in 30 min. Zhou et al. describe a portable instrument for hemoglobin and alanine aminotransferase determination in blood samples based on the measurement of diffuse reflectance from test strips using a portable instrument with a pulsed Xenon arc lamp and an integrating sphere [77].

The second field of application of these types of portable devices is environmental area. In this case, the applications are focused in water analysis and, to a lesser extent, in air analysis. The reason of this is the huge impact that the presence of contaminants or toxic substances in water or air can mean to human health and to environment. Portable analysis devices enable to carry out measurements, in situ and in real time, of water or air which we are in contact with. In these cases, the intervention could be immediate in case that some problem were detected, minimizing drastically the consequences that this problem could entail. Weagant and Karanassios developed a miniaturized, atmospheric pressure, battery operation, self-igniting, planar-geometry microplasma device (MPD) for use with liquid microsamples [78]. This instrument was tested for 9 elements (Cd, Cu, K, Li, Mg, Mn, Na, Pb, and Zn) in bottled-water samples with a precision ranging between 10% and 25%, a linear dynamic range of about one order of magnitude and an LOD of 1.5–350 ng. Pui-ock et al. used a dielectrophoretic field-flow fractionation (dFFF) for detection of eight toxicants having different single or mixed modes of action (acrylonitrile, actinomycin D, carbon tetrachloride, endosulfan, Nnitroso-N-methylurea (NMU), paraquat dichloride, puromycin, and styrene oxide) [42].

From a human and economic resources investment point of view, these two fields of application of portable analytical devices seem to be followed by food analysis area. The developing level of this third application field is still so far away from the clinical and environmental, being considered nowadays as an emerging area of application for these types of devices. The reason of this big difference could be due to the quality controls established in almost every food industry. These quality controls are based on an every time more restrictive legislation, and this one guarantees, in some way, the healthiness of the produced food. However, the consumer information demand has been increased greatly during the last years. In spite of the fact that level of information provided to consumers has grew during the last years, this information is sometimes unsatisfactory and nonexistent in other cases. Consequently, the importance to develop portable devices for food analysis may be not possible for on-line analysis in food industry. From our point of view, the development of these devices should be targeted to the possibility of the consumer will be able to check itself the presence or absence of a compound or family of compounds which could have a direct consequence on its own health. In this way, an automated and polymeric cartridge-based system for the simultaneous detection of multiple antibiotics in milk has been developed [79]. The authors claimed 95% accuracy for multiplexed measurements. Mathies and co-workers have developed a prototype multichannel Mars organic analyzer (McMOA). It is a portable instrument for sensitive microchip capillary electrophoresis (CE) capable of performing CE essays of organic compounds such as amino acid biomarkers, polycyclic aromatic hydrocarbons (PAHs), organic sugars, aldehydes and ketones [80].

4. Matching *lab-on-a-chip* to routine laboratories needs

The convergence of LOC with routine laboratories must be evaluated starting by remarking the basic characteristics of routine laboratories. Thus, firstly, a brief summary of features of routine

laboratories is pointed out, for them to connect to practical LOC approaches.

The main requirements of routine laboratories are, basically, associated to the availability and implementation of robust, rapid, low cost and safety procedures/methodologies; involving durable, cost effectiveness and reproducible equipments providing reliable information. Indeed, analytical results must be representative, traceable and with accepted uncertainty. Method validation is a key requirement, especially for external quality recognition, such as accreditation. From a practical point of view, sample introduction and preparation, separation and detection with calibrated equipments are commonly routine steps.

On the other hand, the main part of the work with LOC are focused on R&D activities such as: (i) design and development of the platform; (ii) association of the physical performance parameters; and (iii) demonstration of application or usefulness. The application, or potential applications, is the future connection with routine work. Application needs an intended use for problem solving and, in such case, transferability to routine laboratories can be planned. Finally, analytical validation for practical use must be systematically checked. Taken into account this sequential process, two key questions can be stated:

- How are the applications of LOC described in the literature?
- What are the main fields of applications?

A general view of literature shows that the main part of applications corresponds to general research, DNA analysis, PCR amplification, clinical diagnosis, and few applications in the fields of environmental and food analysis. Probably, the main types of uses or applications of LOC could be grouped in two categories: (i) analytical studies (investigations) in clinical, biochemical, and bioanalytical fields (“global health”, “point-of-care”, “cell-related operations”, “molecule analysis”, “organism analysis”); and (ii) truly applications addressed to routine analysis in clinical, environmental, food, toxicological, agricultural or industrial fields. With any doubt, some bridges can be established between both groups.

Commercial developments are a good indicator of transferability to real work. Thus, the first commercial microfluidic-based platform for the analysis of DNA, RNA, proteins and cells – known as the 2100 Electrophoresis Bioanalyzer – was launched in 1999 by Agilent. Based on the LOC technology from Caliper Life Sciences, it provides good-quality, quickly and accurately data, and it is an alternative to labor-intensive gel electrophoresis [19,81]. Several companies are now supplying readymade and/or custom-fabricated microchips, which will facilitate application of CE microchip devices. They were mainly used in clinical analysis for diagnosis of cancer, cardiovascular and related diseases, renal markers, neurological diseases, thyroid function, pathogens, immune disorders, diabetes and hereditary diseases.

The last 10 years (since 2000), it can be considered as 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 a decisive proof of this fact, although in many cases the developments remain in the research world, with limited implementation in control or routine laboratories. Mark et al. found that almost 10000 papers have been published over the last 10 years on this topic [82]. According to the ISI Web of Science they currently receive around 40,000 citations per year. Additionally, over 1000 patents have been issued in the USA alone for 10-year period 2000–2009. The application fields of analytical miniaturized devices have been clearly expanded, with a particular impact in the bioanalytical area (cell analysis, single organism analysis, DNA assays, drug screening, protein and peptides analysis) [51].

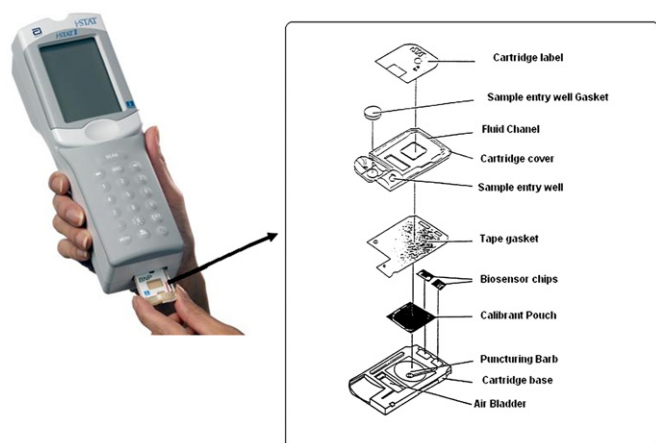


Fig. 3. Photograph of a portable (handheld) blood analyzer i-STAT System (a), and scheme of a disposable sensor-array cartridge used in the i-STAT (courtesy of i-STAT) (b).

In 2000, Abbott Point of Care company manufacture the advanced, portable (handheld) blood analyzer i-STAT System (Fig. 3a), which provides real-time, lab-quality results in minutes, to accelerate the patient care decision-making process. The i-STAT System TM features single-use disposable sensor-array cartridges (Fig. 3b) that perform a broad menu of the most commonly performed diagnostic tests, including cardiac markers, blood gases, chemistries and electrolytes, lactate, coagulation, and hematology. The disposable sensor-array cartridge contains a series of thin-film electrodes microfabricated on a silicon chip. Once the cartridge is inserted into the hand-held meter, the analytical procedure is carried out under an automatic control. Upon completion of the assay, the results are displayed on a liquid-crystal screen, along with the patient-identification number and the time.

Three years later (2003), Auto Genomics presented an automated multiplexing, continuous flow, random access microarray platform Microarray Platform, INFINITI™ System, that integrates all the discrete test processes such as sample handling, reagent management, hybridization, stringency and detection for the analyses of DNA into a totally self-contained system. Some years later, November 2007, ACEA Biosciences and Roche Applied Science entered into an exclusive agreement for the development, supply and distribution for ACEA Bioscience's real-time cell assay technology. ACEA's products are designed to meet the increasing needs of the life science research and drug discovery markets by providing cost-effective, automated, high content, real time detection systems that simplify complex cell-based assay procedures and increase productivity. Other important emergent field is related with the safety and quality of the world's food supply by providing the highest performance biosensors, assay kits and instruments for rapid detection of food pathogens and chemical residues. Therefore, in 2009 was launched HerbiScreen™ as an example of this field, this system uses Novel Biochip to Help Streamline Safety Testing for Herbicides in the World's Food and Water Supply. During the last years, 2010–2011, different companies have provided new range of products. For example, Cellix has provide a range of biochips including the Vena8 Endothelial+™, ideal for studying cell-cell interaction, Vena8 Fluoro+™, ideal for studying cell receptor–ligand interaction and is compatible with confocal microscopy. Finally, it would be interesting to remark that the majority of bio-technology companies have developed a range of products appointed to provide highly uniform whole genome amplification (WGA). Thus, among others, REPLI-g FFPE Kit™ (Qiagen), GenomePlex™ (Sigma–Aldrich), or Illustra GenomiPhi HY DNA Amplification Kit (GE Health Care).

5. LOC: Utopía or reality for routine laboratories?

Undoubtedly, the development of LOC applications, in the course of these last ten years, is clearly multidisciplinary [11,51,71], with research and engineering opportunities straddling across chemical engineering, biology, chemistry, physics, materials, processing science, surface science, information science, and other engineering disciplines. On the other hand, what can be stated about the impact that LOC ambitious concept has had on the “real world”? Has LOC involved a change on the day by day of today's society? In fact, the answers to these questions will be so different depending on the application field considered. No doubt that some actions such as a pregnancy test by yourself at home, to measure the glucose level for a diabetic person, or to in situ checking basic water parameters in a swimming pool, have become a everyday action in our life. Can be expected such type of use of LOC in quotidian life?

The first step in proper prevention and treatment of disease is accurate diagnosis, but diagnostic technologies that are successful in the economically developed world often are difficult to use in developing countries. People who live in these countries frequently cannot afford even modestly expensive tests, and basic infrastructures (i.e., reliable power, refrigeration, and trained personals is often not available). Currently, diagnosis usually requires a trained healthcare worker to recognize symptoms or interpret analyses. By developing effective technologies in health-related diagnostics for developing countries and coupling these technologies to existing communication infrastructures, healthcare in areas without access to trained medical personnel may be possible. According to the World Health Organization, diagnostic devices for developing countries should be ASSURED: affordable, sensitive, specific, user-friendly, rapid and robust, equipment free and deliverable to end-users [83].

In this sense, Chin et al. integrated new procedures for manufacturing, fluid handling and signal detection in microfluidics into a single, easy-to-use point-of-care (POC) assay that faithfully replicates all steps of ELISA, at a lower total material cost [84]. These authors performed this ‘mChip’ assay in Rwanda on hundreds of locally collected human samples. The chip had excellent performance in the diagnosis of HIV using only 1 mL of unprocessed whole blood and an ability to simultaneously diagnose HIV and syphilis with sensitivities and specificities that rival those of reference benchtop assays. Unlike most current rapid tests, the mChip test does not require user interpretation of the signal. This demonstrates an integrated strategy for miniaturizing complex laboratory assays using microfluidics and nanoparticles to enable POC diagnostics and early detection of infectious diseases in remote settings.

In 2004, the Sia et al. [85] introduced “POCKET immunoassay”, a portable and battery-operated microfluidic-based system designed to address the need for simple diagnostic devices in developing countries. POCKET could run quantitative immunoassays in the field with the same sensitivity as bench-top enzyme-linked immunosorbent assays (ELISA), but in a fraction of the time and at a fraction of the cost.

Diagnostic devices made of patterned paper—which called microfluidic paper-based analytical devices (μ PADs), also known as a lab-on-paper technology, are a new platform designed for ASSURED diagnostic assays. These systems combine some of the capabilities of conventional microfluidic devices with the simplicity of diagnostic strip tests [86,87]. When fully developed, μ PADs may provide bioanalyses that are more rapid, less expensive, and more highly multiplexed than current analyses. They only require small volumes of fluid and little or no external supporting equipment or power because fluid movement in μ PADs is controlled largely by capillarity and evaporation. These devices were recently developed by Martinez et al. [86]. The idea was to make microfluidic devices out of paper instead of plastic or glass. Over the past 6

years, many developments have been made in the emerging field of μ PADs [88–98]. Reviewing the development of these devices in the Whitesides's [86–88,92,95,96,98,99], different groups can provide some insight into the future of the field and encourage scientists from a variety of backgrounds to contribute their expertise toward further development of useful μ PADs.

The argument for developing low-cost diagnostic devices is still as compelling as it was when the project started: millions of people die every year, mostly in developing countries, from preventable or treatable diseases [100] and portable diagnostic devices could help save many of these lives. μ PADs have not yet made it into the field and have not yet saved any lives, but they are advancing steadily toward this goal. While μ PADs were first developed as diagnostic devices for use in developing countries, actually, they were designed to various applications including health diagnostics [101–103], environmental monitoring [94,92] as well as food quality testing [104].

As it has been reported before, there are diverse application areas for LOC, but it is undoubtedly bio-analytic field, with DNA analysis and PCR amplification, followed by clinic field, with “point-of-care” devices, which have entailed a practical acceptance in routine laboratories. Succeeding these two dominant application fields would be environmental analysis following in last place food analysis; both of them can be considered emergent fields. So, it would be important to evaluate the different level of incorporation of LOC, according to the fields of application, to routine laboratories. In this way, it is unquestionable the great impact that amplification DNA or cells analysis and PCR amplification chips have had over the working order of the laboratories in charge of this type of analysis. Thus, working protocols have been changed, modifying fundamental parameters that used to imply this type of analysis, especially required time to be carried out. Consequently, in the market it can be found, among other commercial devices, single-use packs, used for performing cell-based assays on the xCELLigence™ system, from ACEA Bioscience; the GeneXpert™ System, from Cepheid, which is the only system combining on-board sample preparation with real-time PCR amplification and detection functions for fully integrated and automated nucleic acid analysis; or Genalysis™ platform, from DNA electronics, which is capable of delivering real-time, disposable, accurate on-the-spot tests for any target nucleic acid sequence (DNA/RNA).

The second application field nowadays is the clinic diagnosis, with the well-known as “point-of care” devices. There are many laboratories, or pharmaceutical companies, which have bet on this type of devices. The general tendency has been firstly to focus on the development of a “universal” measure device which can be used for several applications by using a wide range of chips. Development and expansion of this range of chips with different applications has been, in general, the second stage of the project. Among this devices it would be i-STAT System™, from Abbott Point of Care, which performs a broad menu of the most commonly performed diagnostic tests, including cardiac markers, blood gases, electrolytes, lactate, coagulation, and hematology; or Piccolo™ X-press, from Abaxis, a compact, portable clinical chemistry system designed for on-site patient testing. The different panels are configured to aid in disease diagnosis or monitor disease treatment such as “basic metabolic panel”, “liver panel” or “renal panel”. Also, Epoc™ point of care blood analysis system, from Epocal, which uses Smart Card technology. Epoc test cards currently in development include additional blood chemistries, hemostasis, hematology and immunoassay panels.

Finally, following from a long distance, LOC has been adapted to the environmental and food applications, but with very few applications. However, it can be found some commercial products, as examples reported before represented by HerbiScreen™, from Biodetection Instruments for detection of herbicides; or Mericon

Pathogen Detection Assays, from QIAGEN, including kits covering all segments of food testing, including pathogens, genetically modified organisms, allergens and plant and animal identification.

The justification to this short implementation of LOC to environmental and a food field is complex, and the explanation to this fact could be finally due to more than one factor. In one hand, it is widely known the huge economic and time investment necessary since an idea spring up until this idea becomes a reality. An investment of these characteristics is not easy to be afforded by companies, being the pharmaceutical industry in clear superiority, especially over environmental or food industry. The other reason could be the different demand of analysis in the different fields. Overload of hospital due to the centralization of clinic analysis, together with in most of cases clinic parameters ordered were the same, have been the main reason lab-on-a-chip had an overwhelming acceptance in the bioanalytic and clinic routine laboratories. With respect to food field, until now the information required was limited to the parameters that industry fixed itself according to the established legislation. However, the information required by consumers has been rocked as a consequence of the new life habits and the emergence of new alimentary problems, such as allergens or food intolerance. It is a golden rule that market always is adapted to consumer demand. For this reason, it is expected that the next years the food field will undergo an important transformation with respect to routine analysis laboratories.

Some innovations such as array biochips and microfluidic-based biochips (lab-on-a-chip) devices are becoming a commercial reality. These biochips are transitioning out of the academic and pharmaceutical research realm to more commercialized applications for routine analysis, such as medical diagnostics, environment and agri-business, and pathogen detection. Early biochips were based on the concept of a DNA microarray designed for the purpose of accelerating genetic research. Now, biochips are not limited to DNA analysis. Protein biochip array, antibody biochip array and chemical compound biochip array are also available to for disease screening, diagnosis and monitoring [23]. From an economical point of view, biochips were developed and commercialized in the nineties.

Since then, market has offered a dramatic growth and, hence, seen an influx of players vying fiercely for a share of this market. The global biochips market is studied by the types and the key applications. According the BBC research report concerning global chip markets [105], the total market for biochips was \$2.4 billion in 2008 which increased to \$2.6 billion in 2009. This is expected to grow at compound annual growth rate (CAGR) of 17.7% to reach \$5.9 billion by 2014. The DNA microarrays market was nearly \$1.2 billion in 2008; this further increased to \$1.3 billion in 2009, and it is projected to reach \$2.7 billion in 2014, for a 5-year CAGR of 15.2%. Sales in the LOC market amounted to \$755 million in 2008 which increased to \$817 million in 2009. This is projected to increase to \$2.1 billion in 2014, for a 5-year CAGR of 20.9%. Based on this BBC Research source, Fig. 4 summarizes global value (\$ millions) of biochip products by type, since 2007 until 2014 [105]. It can be seen that DNA microarrays and LOAC are the widest fields that dominate the market today; protein and emerging microarrays fields are also of high potential. Some selected companies with Biochip programs are presented in Table 1 [106]. Most of these companies are pharmaceutical companies. However, in our point of view, outside the human medical sector we see a huge potential for pocket-sized labs in veterinary medicine and food industry. Might be these industries, with lower economic power, can profit from the enormous investment of pharmaceutical industry has done in this area, achieving to establish the main principles at both, scientific and technical point of view. Because of their integrated nature, biochips are also ideal for environmental monitoring, as they can be portable and provide selective, sensitive and rapid responses in real time.

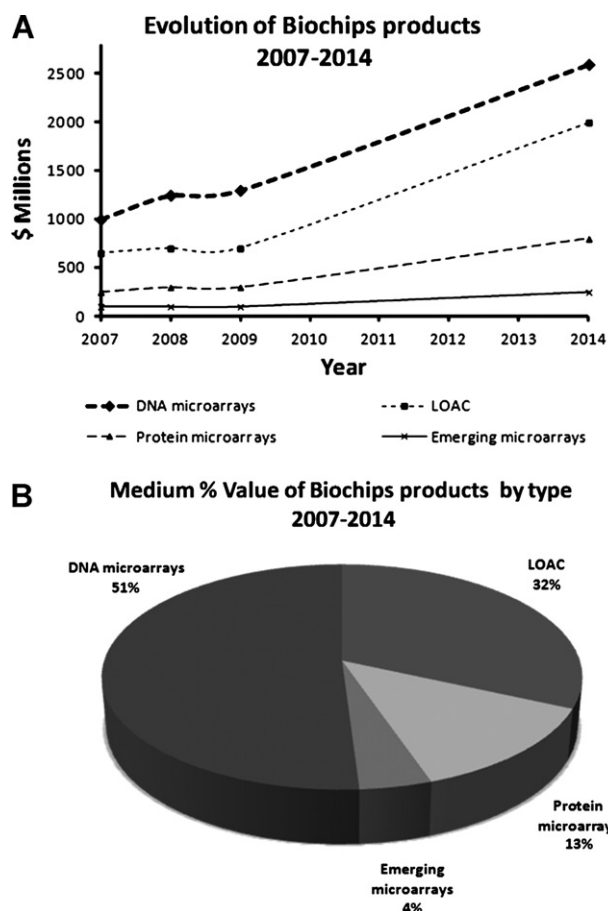


Fig. 4. Global value (\$ millions) of biochip products: (A) evolution by type since 2006 until 2013; and (B) percentage of each type biochip from the total value.

Thus, Brennan et al. developed a fluidic biochips for rapid screening test for potential chemical contaminants in water samples [107].

6. Conclusions and trends in LOC

As described in the above section, they are numerous reports of clever, microfabricated devices, and applications that purport to provide the promise of LOC benefits. Yet for the most part, we have seen only limited commercial success on the part of any company that has been bold enough to venture into the market offering LOC products. Although exciting to contemplate, “chips” have not been widely accepted yet in the commercial area. Customers become enthusiastic about them at meetings and from novel literature reports of LOC developments. Yet LOC techniques have not demonstrated the compelling, cost-effective benefits required to displace current technology or workflow. Nor has it shown the ease of use needed to induce users to change. The major benefit claims of minimum sample requirement and solvent and reagent savings from LOC approaches seem encouraging. Ultimately, however, they are not so high a priority for most practitioners, who are not so constrained by considerations of sample waste that the benefit of acquiring LOC technology would clearly outweigh the burden of doing so. As for other waste, in most cases, scaling-down approaches such as micro- and nano-LC, easily reduce solvent and reagent consumption to acceptable levels. Perhaps the mission to “go green” will help support LOC efforts, but for the foreseeable future, environmental considerations do not constitute a compelling reason for most to switch to chip-based approaches.

Table 1
Selected companies with biochip programs.

Company	Biochips products
<i>Biacore</i> : Is a global supplier of systems for protein interaction analysis, an area of importance for scientists in the academic, pharmaceutical, biotechnology, and diagnostic markets	Sensor Chip C1 -Sensor Chip CM3 -Sensor Chip CM4 -Sensor Chip CM5 -Sensor Chip L1
<i>AVIVA Biosciences</i> : Develops enabling drug discovery and development technologies by combining biochips with cell biology.	-Multiple Force Biochips
<i>Advalytix</i> : Main focuses is the miniaturization of diagnostic and biological tests for research and development.	-Programmable Biochips
<i>Alpha Innotech</i> : Develops, manufactures, and markets digital imaging and detection systems for the life science research and drug discovery markets	-Aplazcan and ArrayVision
<i>Applied Precision, Inc.</i> : Provides comprehensive microscopy imaging solutions to help scientists address disease-related questions for cellular and sub-cellular analyses beyond the limitations of conventional methods.	-ArrayWoRx Auto Biochip Reader -ArrayWoRx Biochip Reader
<i>Berthold Technologies</i> : contribute to the development of sensors, detection systems and instruments.	-SpotLight LB 990 Biochip Reader
<i>Ciphergen Biosystems</i> : Is a leading provider of enabling tools and collaborative services in the emerging field of protein-based biology research, known as proteomics	-Au chip ProteinChip Arrays -CM10 ProteinChip Arrays -IMAC3 ProteinChip Arrays -IMAC30 ProteinChip Arrays -SAX2 ProteinChip Arrays
<i>CombiMatrix</i> : Is a biotechnology company, patented electrochemical manufacturing process utilizes standard semiconductor technology, proprietary software, and chemistry to build arrays of materials molecule by molecule.	-Hyb & Seq
<i>EPFL</i> : Develops novel engineering methods that bypass the limitations typically associated with classical micro/nanofabrication.	-SU-8 Biochip
<i>Fluidigm</i> : Develop, manufacture, and distribute systems composed of IFCs, instrumentation, and consumables, enabling new efficiencies in science and industry.	-TOPAZ Screening Chips
<i>Atomics</i> : Is focusing in electro- and nano-mechanical diagnostic point of care technologies.	-Atopic Biochip -Food Biochip -Specific Allergen Biochip -Total IgE Biochip

Good reasons nonetheless abound for embracing LOC developments for future implementation, commercialization, and success. Despite the merits of the current sample analysis capabilities, many deficiencies in the analytical strategies and workflow continue to plague us: sample transfer losses, extra-column variance in chromatographic separations, expenses related to excess solvent and reagent disposal, and detection limitations associate with sample dilution—to name but a few. Although the modern sample preparation-analysis procedures coupled with MS work fairly well, there is always room for improvement. Finally, for many laboratories, acquiring personnel whose skill levels are equal to performing experiments using complex equipment is challenging. LOC techniques could lessen the challenge by integrating some procedure steps on an automated chip.

Table 2
Critical view of lab-on-a-chips in the analytical field.

(A) Potential advantages
Related to the miniaturization
Compatibility with micro/nano sensors and miniaturized detection devices
Portability
In situ/in vivo measurements
Capabilities for working with single small entities
Related to the mode of operation
Very low sample and reagent consumption
Related to the analytical properties
Short time of analysis
Separation of compounds with good resolution (multiparametric analysis)
(B) Disadvantages
Microfabrication requirements
Manual introduction of sample/reagents (low level of automation)
Clean (conditioning) steps should be included to assure reproducibility
Lack of representativeness can be produced because the very small plug of sample
Experimental conditions must be systematically controlled for analytical applications
(C) Present challenges
Reproducibility in the fabrication
Transfer to control/routine laboratories (robustness and transferability)
Miniaturization of the associated equipments

With respect of biochips, the future trend is heading toward smaller, cheaper, and faster integrated lab-on-a-chip devices. However, despite all the advances in the development of miniaturized sensing and analytical components for use in a variety of biomedical and clinical applications, the ability to assemble and interface individual components in order to achieve high level of integration in complete working systems continue to pose daunting challenges for industry as a whole [108]. The future of the biochips industry will depend heavily on the collaboration of multiple disciplines such as the biological, physical, chemical, and engineering sciences to bridge the gaps in technology and establish industry standards to support mass commercialization.

Given that human nature disposes us toward risk averseness or laziness, we often find insufficient reason to change our ways without compelling grounds to do so. Thus, commercial success of LOC products that integrate MS will await a day when such products prove themselves easy to use, robust, reliable, and economically viable. The incorporation of lab-on a chip to routine laboratories to these others emergent areas, as most of matters in our society, it is a question of supply and demand. The increase of information demand in these two fields is now a reality, and it is known this demand cannot be satisfied by the present methods. For this reason, according to normal society development, the incorporation of lab-on-a-chip in routine laboratories of environmental and food analysis is only a question of time, and in our point of view, it will be in a near short term. For this evolution/revolution a clear view of the present LOC situation, such as Table 2 summarizes, should be taken into account. Interesting advantages related to miniaturization, mode of operation and analytical features can be stated. Probably, those associated to miniaturization/simplification of the measurement process are very valuable and they provide solid arguments to a successful future of LOC (compatibility with miniaturized detection devices, portability, in situ/in vivo measurements, capabilities for working with small entities such as cells, and micro-organisms). On the other hand, disadvantages must be taken into account, studying possible alternatives for solving these problems or limitations: simplification of the microfabrication process, automatic integration of sample introduction, integration of cleaning steps with analysis steps, systematic control of experimental conditions (good reproducibility to assure acceptable uncertainty), and the development of protocols to assure sample representativeness. Together these disadvantages, some challenges seem to be evident

for the successful incorporation of LOC in routine work such as: the development of robust methods associated to the hardware (platforms, chips, etc.), and the consequent validation to be transferred to routine laboratories; and the systematic miniaturization of the associated equipment (power suppliers, external units and connections, etc.). But, at the same time, it is necessary to recognize that although transferable LOC methods could be available in the near future, well established control/routine laboratories working in food, environmental or industrial fields, are not easily open to adopt other methods than official, standard or reference methods. Therefore, and probably, the most common “analytical niche” for LOC could be for non-routine analytical work, work involving small entities, or as analytical tools for research work in other disciplines.

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