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Microsystem Engineering of Lab-on-a-chip Devices

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Library of Congress Card No.: applied for

British Library Cataloguing-in-Publication Data
A catalogue record for this book is available from the British Library.

Bibliographic information published by Die Deutsche Bibliothek
Die Deutsche Bibliothek lists this publication in the Deutsche Nationalbibliografie; detailed bibliographic data is available in the Internet at <http://dnb.ddb.de>

Printed in the Federal Republic of Germany
Printed on acid-free paper

Typesetting K+V Fotosatz GmbH, Beerfelden
Printing betz-druck gmbh, Darmstadt
Bookbinding Litges & Dopf Buchbinderei GmbH, Heppenheim

ISBN 3-527-30733-8

Contents

Preface

We live in a world that is influenced by technological developments. One of the clearest examples of this is microtechnology. The use of microtechnology to miniaturize and functionally integrate electronic components has changed our world and hardly any facet of our lives is not in some way affected by microelectronics. Building on the experience of microelectronics research and industry we have started to apply microtechnology to chemistry and biochemistry. We stand to gain many advantages including improved performance, portability, and reduction of cost. The application of microtechnology to chemical and biochemical analysis is a very multidisciplinary topic which needs input from scientist and engineers with different backgrounds. This book combines the experience of a group of engineers, chemists, physicists, and biochemists who are applying microtechnology to chemical and biochemical analysis at the Mikroelektronik Centret (MIC) at the Technical University of Denmark (DTU). The various stages in the development of such microsystems are described in this text book: from concept to design, to fabrication, and to testing. There is little doubt in the international research and industry community that the application of microtechnology to chemistry and biochemistry will revolutionize our lives in a way that is comparable to what we have seen with microelectronics. Our aim with this book is to allow a broad range of scientists and engineers to get interested and familiarized with this very exciting topic.

Lyngby, July 2003

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1
Introduction

Pieter Telleman

1.1
Learning from the Experiences of Microelectronics

Try to think back to the time that your parents were your age and imagine the technological developments that have taken place since then. Sometimes it is hard to imagine that only 2 decades ago personal computers, mobile phones, compact disks (CD) players, and digital video disks (DVD) players did not exist. What made these technological developments possible? One of the major contributing factors is microelectronics. The first breakthrough from electronics to microelectronics was the invention of the transistor in 1947 at Bell laboratories. Transistors provided a better, cheaper alternative to mechanical relays, which were the standard electronic component for switching and modulating electronic signals. With improving semiconductor technology, transistors became progressively smaller, cheaper, and better. A second breakthrough was the introduction of the integrated circuit in 1959, by which numerous transistors and other electronic components together with the necessary wiring were organized on a thin silicon disk or wafer. In 1965, only 4 years after the introduction of the integrated circuit, Gordon Moore predicted an exponential growth of the number of transistors in an integrated circuit (Moore's Law). Although the pace has slowed down a bit in recent years, experts agree that the current rate of a doubling every 18 months will continue at least for 2 more decades. If we should summarize the process that made microelectronics so successful, we could say that it was the combination of miniaturization, i.e., microfabrication of transistors and other electronic components, and functional integration, i.e., the organization of many different miniature electronic components to form integrated circuits with complex functions. Since the application of miniaturization and functional integration to electronics, the same strategy has been applied to a range of other disciplines, e.g., mechanics and optics. One example of a microelectromechanical system (MEMS) is the accelerometer. The deployment of airbags in cars depends on signals from a number of accelerometers, i.e., miniaturized mechanical sensors that measure the *g* forces on the car. Other examples of MEMS are pressure sensors and microphones. The promise of faster and better data transfer offered by optical communication has resulted in the application of microtechnology to develop microstructures for the manipulation of light, e.g., micromirrors and optical switches.

In 1979, S.C. Terry et al. presented "A gas chromatographic air analyzer fabricated on silicon wafer using integrated circuit technology" [1]. This was the first publication that discussed the use of techniques borrowed from microelectronics to fabricate a structure for chemical analysis . The introduction of the concept of micro total-analysis systems (μTAS) by Manz and coworkers in 1990 [2] triggered rapidly growing interest in the development of microsystems in which all the stages of chemical analysis such as sample pre-preparation, chemical reactions, analyte separation, analyte purification, analyte detection, and data analysis are performed in an integrated and automated fashion. The aim of this textbook is to provide you with a comprehensive understanding of the concept of μTAS. We will introduce you to microfluidics, i.e., the manipulation of small amounts of reagents and sample on microchip, simulation and modeling of microfluidics, fabrication of microsystems for chemical analysis in silicon, glass, and plastics, packaging of microsystems, and several examples of chemical analysis in microstructures.

1.2 The Advantages of Miniaturizing Systems for Chemical Analysis

Why is it that, when the concept of μTAS was introduced in the early 1990s, it attracted so much interest from the scientific and the industrial community? It was because the conventional approach to chemical analysis can no longer meet all the requirements that many applications demand. Let us look at some of these requirements and see how μTAS can offer unique solutions.

With rapid developments and growing interest in, e.g., medicine, drug discovery, biotechnology, and environmental monitoring, we have become more and more dependent on chemical analysis. Traditionally, chemical analyses have been performed in central laboratories because they require skilled personnel and specialized equipment. However, the trend is to move chemical analysis closer to the 'customer'. Some examples are pregnancy tests, blood glucose concentration tests for diabetes patients, and analysis of soil and water samples. These chemical test kits can be acquired off the shelf and can be used in the home by persons with no special training in chemistry. This trend of decentralization of chemical analyses is expected to continue. For this to happen we need to make analytical equipment smaller and thus portable, easier to operate, and reliable. The results of the chemical analyses must be processed so that it is easy for the user to interpret. The concept of μTAS builds on performing all the necessary steps that are required for a chemical analysis on a miniaturized format and thereby offers portability. Because the microfabricated components in a μTAS can be operated with very low power consumption, battery-operated analytical equipment opens up the possibility of performing chemical analyses in the field independent of a power grid. Automation of the entire chemical analysis process and data processing is also part of the μTAS concept. In its extreme case μTAS can be represented as a black box where the user needs only to apply the sample and push a start button

to perform the chemical analysis and retrieve the results. Microfabrication allows us to reproduce the same carefully designed μTAS many times with the same specifications. When care is taken to address reliability at the stage of designing a μTAS, reliability can be warranted for large batches. At the heart of each μTAS is a chip in which fractions of microliters of samples and reagents are moved around with very high accuracy. Traditionally chemical analyses are performed by mixing milliliters of samples and reagents in conventional test tubes and analyzing the product in an analytical instrument, e.g., a spectrophotometer. Especially when the samples and reagents are in short supply or very expensive, μTAS offers a significant decrease in costs by dramatically reducing the volume of samples and reagents that are needed to perform a chemical analysis. We already mentioned that once a μTAS has been successfully developed, it can be reproduced faithfully in very large numbers. This opens up the possibility of processing samples in parallel, which is very useful when the same chemical analyses must be performed many times over. This is exactly what drug discovery is about. A drug candidate often needs to be identified from a pool of many thousands of samples by performing a particular chemical analysis on each sample (this process is referred to as high-throughput screening or HTS). Today HTS is implemented by performing the chemical analysis in microtiter plates in combination with robotic handling of the samples and reagents. The possibility μTAS offers of parallelizing chemical analyses is seen as an interesting alternative to the use of microtiter plates and will eventually allow an increase in throughput.

Often, we want to know how the concentration of an analyte changes in time, i.e., online monitoring. It is better to continuously monitor the concentration of glucose in the blood of a diabetes patient than to measure the glucose concentration once every so many hours. Continuous analysis of ammonium in wastewater is more valuable for controlling a sewage-treatment plant than a measurement only 2 or 3 times a day. With conventional methods of chemical analysis is it difficult to implement online chemical analyses. Handling and processing of the sample is, at least in part, done manually and often in specialized laboratories. But with μTAS, we can bring the chemical analyses close to the place where they need to be performed, independent of a laboratory and laboratory personnel. Sample handling and processing, the chemical analysis, and data processing are integrated in μTAS, which makes it very well suited for online measurements.

The advantages of μTAS can be summarized as follows: μTAS offers portability, reliability, reduction of sample and reagent consumption, automation of chemical analysis, high-throughput screening, and online analysis. Keep in mind however, that μTAS has been around only since the late 1980s and that a much research and development still has to be performed in order to fully benefit from all its advantages. Several issues that are essential to the widespread use of μTAS have received little attention so far. The most prominent of these issues are interconnection and packaging. Regardless of how skilled we are in designing and fabricating μTAS, the chip at the heart of the μTAS must be interfaced to the macroworld of the user. For μTAS, this requires fluidic, mechanic, optical, and electronic interconnections. Furthermore, μTAS must be packaged so they can be handled safely

without damaging the delicate microstructures on the chip. Both issues must be dealt with to allow for successful commercialization and thereby wider use of the technology.

1.3 From Concept to μTAS

When you received this book you most likely started to flip through the pages to see what you can expect in the coming days or weeks. And you discovered that this book addresses a wide range of subjects that belong to many different disciplines, including physics, chemistry, and computer sciences. μTAS is a truly multidisciplinary activity that requires input from scientists having many different backgrounds.

The process of developing a μTAS consists of several discrete steps, starting with determining the specifications for the μTAS (Fig. 1.1). These specifications depend mainly on the nature of the chemical analysis and must answer questions such as: which reagents are used? what are the reaction kinetics? at what temperature are the reactions performed? what means of detection will be used? what is the desired range of detection? what is the required limit of detection? The chemistry in turn determines what material can be used for fabrication of the μTAS, for example: should it be transparent? are the reagents aggressive? is the μTAS intended for single use or multiple use? Inherent to combining mechanics, fluidics, optics, and electronics in μTAS is the formation of interfaces between these media. One must be aware of the fact that the sensor function of μTAS is actually based on the interfaces between 2 or more media, e.g., for absorption measurements you need an interface between light and a chemical. The interface of μTAS and the user, i.e., interconnection and packaging, must be also considered during

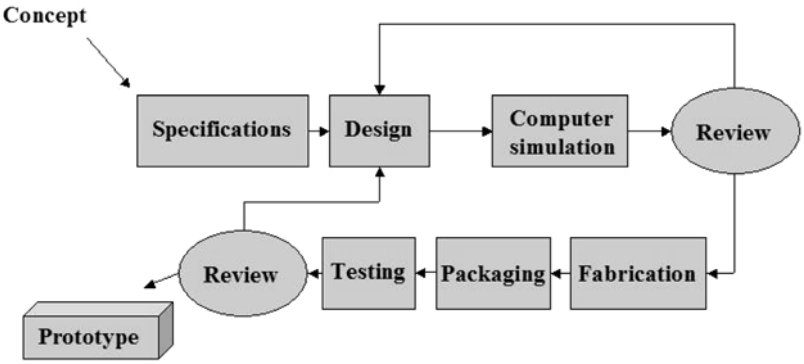


Fig. 1.1 From concept to μTAS. The successful development of a μTAS involves a number of discrete steps: specifications of the chemical analysis, design, modeling to evaluate performance, fabrication, and testing. Reviews of the modeling and test results enable optimization of the performance of the μTAS.

the specification phase. Defining the specifications for μTAS is a process that should involve all project members because it affects the overall μTAS performance.

With the specifications in place, the next step is to design the μTAS. Design constitutes the most important block in the flow sheet from μTAS concept to prototype and is discussed in more detail in chapters 3 and 4. It is here that considerations of μTAS concept, definition of interfaces, and specifications are translated to a fabrication plan. Developing a sequence of process steps for individual μTAS components, e.g., micropumps, is challenging in itself, but aiming at μTAS, where the entire process sequence involves a variety of integrated components, raises questions of process sequence and compatibility. How does one combine, from a process point of view, for example, microfluidic components with optical components without loosing the properties of the individual structures due to process incompatibility somewhere along the way? Is the choice of a particular process sequence compatible with demands for packaging? One of the first steps in establishing a complete and effective μTAS platform must be the categorizing of all process steps that are involved in making individual components, investigating process compatibility, and finding alternative processes or process sequences in cases of incompatibility. Design is in many ways a matter of experience and intuition and, with a design that satisfies the demands of the different partners involved, it is in principle possible to start fabricating the μTAS. However, depending on the complexity of the design, it is often very difficult to predict the performance of the μTAS intuitively. In these cases computer simulations may provide a means to study the performance of a μTAS prior to fabrication.

Computer simulations can significantly shorten the possibly long process of μTAS design, fabrication, and testing. The behavior of individual components, as well as the interplay between integrated components, can be predicted by computer simulations. By including a review step after computer simulation, structures can be optimized for their geometry and operational parameters based on the simulation results prior to actually fabricating the components or devices. This rational approach constitutes a significant improvement over the approach in which computer simulation is omitted and structures are optimized by numerous rounds of fabrication and testing. Important aspects of computer simulations are addressed in chapter 5. Key to the development of μTAS is microfabrication: the fabrication of structures down to micrometers in size. Aspects of microfabrication in silicon, glass, and polymers are discussed in chapters 6, 7 and 8. The explosive growth of microelectronics has led to a wide range of microfabrication tools for silicon, and consequently, much higher levels of experience and expertise exist for working with silicon as a material for microtechnology. Silicon presented an obvious choice as a material for the microelectronics industry due to its semiconductor properties. Few materials can surpass silicon when it comes to fabricating microstructures: silicon is suitable for the fabrication of electronic, mechanical, and optical components and thereby allows for high levels of functional integration. However, the superiority of silicon as a material for μTAS is debatable because the chemical stability of silicon is not very good. In fact, many of the microfabrication

methods available today are based on the controlled removal of silicon by chemical treatments. Although the surface of silicon can be treated to withstand harsh chemical environments, other materials may be more suitable for certain applications. Another important argument for investigating alternative materials is the relatively high cost of silicon, especially in applications where μTAS that have been in contact with biohazardous materials like blood are discarded after a single use. For these reasons polymers and glasses offer interesting alternatives to the use of silicon for μTAS. Because the use of polymers and glasses for mechanical, optical, and electronic components is still very much under development, fabrication of these materials carries with it concessions as to the level of functional integration that can be achieved. Hybrid solutions, in which microstructures of different functions and fabricated of different materials are assembled to make up a complete μTAS, will most likely arise.

With fabrication complete, structures must be tested in the laboratory to assess to what extent they live up to the previously defined specifications and how well computer simulations were able to predict the performance of the μTAS. When the device does not perform according to the specifications, all aspects downstream from the specifications need to be reconsidered. Modeling tools will have to be modified if they cannot predict the behavior of μTAS accurately enough.

As mentioned earlier, the aim of μTAS is a complete integration of all necessary steps for conducting a complete chemical analysis. Depending on the duration and complexity of the entire process of design and fabrication of μTAS, you can imagine that the final μTAS can be very expensive. In applications where the μTAS offers a significant improvement over conventional chemical analysis techniques and where the expected useful lifetime of the μTAS is long, the potential high cost of μTAS may not be the decisive factor that prevents its use. However, in applications where the μTAS is discarded after a single use, the cost of μTAS is very important. In some cases we may be simply unable to realize a true μTAS because we lack the technology to integrate certain essential components, e.g., lasers. The formal concept of functional integration in μTAS and all the accompanying advantages must therefore be balanced against complexity, cost, and feasibility. Undoubtedly we will see many examples of μTAS that result from the assembly of a microfabricated chip with conventional, possibly miniaturized, components, e.g., pumps, light sources, electronics. The assembly of these hybrids between microtechnology and conventional technology can be adjusted so that the level of integration makes sense for the individual application. With hybrid technology, you can discard certain parts of the hybrid while keeping expensive functional units like pumps and light sources.

At the time of writing this textbook, the commercial market for μTAS-based products is still rather small. However, market research reports predict consistent growth in the global market for μTAS-based products. These reports also agree that chemistry and the life sciences continue to be the major users of microsystem technology. With the anticipated future technological developments in chemistry and the life sciences, it is clear that microtechnology in general and μTAS specifically will play an essential role in these developments. Many fundamental

problems still need to be addressed to allow for the routine application of μTAS in chemistry and the life sciences, the most pertinent being interconnection and packaging of the μTAS to allow handling by the operators. It is likely that answers to these 2 factors will determine the ultimate commercial success of μTAS. Interconnection and packaging are discussed in detail in chapter 9. The need for a paradigm shift in chemical and biochemical analyses to satisfy the needs of research and industry is, however, so large that solutions to these problems will undoubtedly be found and μTAS will be a part of our future.

1.4 References

1 Terry, S.C., Jerman, G.H., Angell, J.B. (1979) *A gas chromatographic air analyzer fabricated on a silicon wafer.* IEEE Trans. Electron Devices. ED-26, 12, 1880–1886.

2 Manz, A., Graber, N., Widmer, H.M. (1990) Miniaturized total chemical analyses systems. A novel concept for chemical sensing. *Sens. Actuators, B Chem.,* B1 (1–6), 244.

2
Clean Rooms

Daria Petersen and Pieter Telleman

The functional components in μTAS can have dimensions down to micrometers, and particles in the atmosphere that contaminate such components can completely destroy the function of a μTAS. The concentration of particles larger than 0.5 μm in the air of a classroom or office building can be as high as 50 million particles per cubic meter. To avoid contamination of wafers with particles, a special laboratory is needed: a clean room. In a clean room, air-borne particles are removed by continuously filtering the air through a high-efficiency particulate air filtering (HEPA) system. HEPA filtering systems are a class of air filters that retain close to 100% of particles as small as 0.3 μm (Fig. 2.1).

Clean rooms include several sections that have different requirements for cleanliness. The air in areas where wafers are handled must be kept as clean as possible, but the air quality in service areas, which contain the bulk of the equipment, is less critical. To prevent contaminated air from entering those areas where air quality is most critical, the air pressure in these areas is kept slightly higher. To minimize the circulation of particles in the clean room, filtered air enters the clean room through the perforated ceiling and is removed through the raised, perforated floor. The air flow regime in a clean room is laminar, i.e., turbulent flow is absent, to prevent particles from travelling through the clean room so they can be efficiently and rapidly removed. The velocity of the laminar flow in the clean room that is used at the Mikroelektronik Centret at the Technical University of Denmark is about 0.4 m s^{-1}, the intake of fresh air into this clean room is about 30 000 m^3 h^{-1}, and the air flow inside this clean room is about 130 000 m^3 h^{-1}. Air inside the clean room is recycled as much as possible, but exhaust air from the equipment, fume hoods, and wet chemical benches is not recycled. To avoid contamination, equipment is placed in clean rooms so as to prevent return air paths. Only the purest starting materials and processing chemicals should be used in a clean room, and equipment in the clean room must be cleaned periodically to maintain the cleanest environment possible. Clean room users are responsible for daily cleaning and releasing as few particles as possible. For example, one has to avoid quick movements in the clean room so as not to disturb the laminar air flow pattern. Talking to colleagues in the clean room must be kept to a minimum, because talking generates many particles and aerosols. Smoking within half an hour before entering a clean room is forbidden, to reduce the emission of

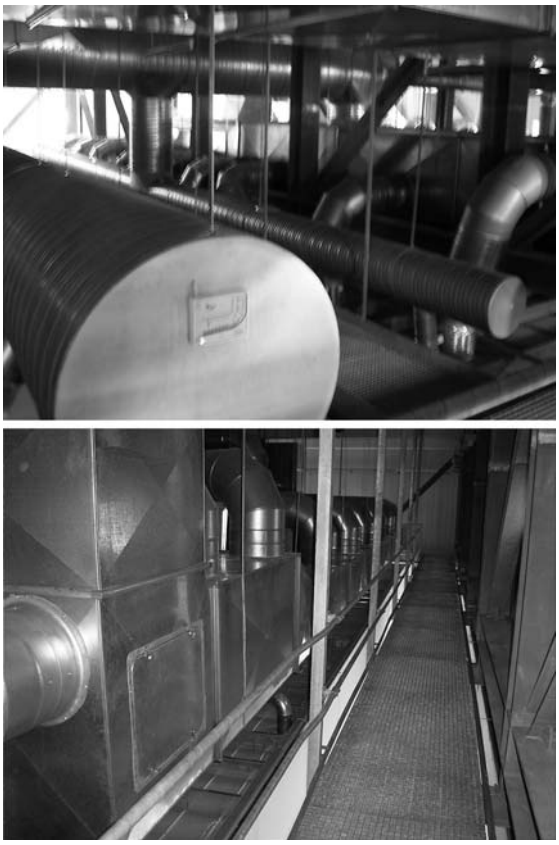


Fig. 2.1 Ventilation system of the clean room at the Mikroelektronik Centret at the Technical University of Denmark, which contains HEPA filters to remove particles from the air.

small smoke particles from the lungs. No form of makeup is allowed while working in the clean room, since most makeup is based on particles. Humidity and temperature in clean rooms are kept constant at 45% relative humidity and 21 °C, to maintain consistent experimental conditions and create a good working environment.

Federal Standard 209E of the USA describes basic design and performance requirements for different classes of clean room. This standard is used for most clean rooms. Classification of a clean room by this federal standard sets the maximum number of particles larger than 0.5 μm in each cubic foot of air. For example, a class-1000 clean room has fewer than 1000 particles larger than 0.5 μm per cubic foot. For the microfabrication of μTAS, class-1000 or class-100 clean rooms are usually sufficient.

People working in the clean room are the main source of contamination. A person can shed as many as tens of thousands to tens of millions of particles per

Fig. 2.2 A cleanroom suit prevents contamination of the clean room by the user.

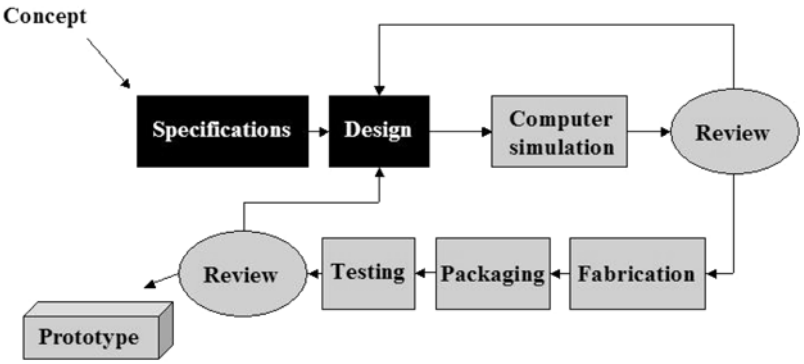
minute. To prevent the bulk of these particles from entering the clean room, individuals working in the clean room have to put on a cleanroom suit before they enter the clean room. A cleanroom suit consists of a cap, a coverall, and boots. In addition to the cleanroom suit, disposable gloves are used, which means that the suit covers pretty much the entire body except for the face (Fig. 2.2).

Cleanroom suits are cleaned frequently and made from materials that emit very few particles. Microfabrication involves extensive use of many toxic gases and other dangerous chemicals and therefore many safety precautions are in force in a clean room. Keep in mind that the cleanroom suit is there only to protect the clean room from the user and not the other way round. Cleanroom suits offer no protection from chemicals. When handling chemicals, additional protection is offered by face shields and special chemical-resistant gloves.

A clean room is a complex and potentially dangerous laboratory environment, which requires that everyone who will work there must be trained extensively in proper cleanroom behavior and proper safety routines.

3
Microfluidics – Theoretical Aspects

Jörg P. Kutter and Henning Klank



When we think about flows in everyday life and our typical experiences with them, we think of a river flowing down its bed, water flowing out of a faucet, or a beverage filling a glass. We might even think of blood flowing through our veins and maybe ink flowing out of a pen (especially when more ink is coming out than is supposed to). It is much less likely, however, that we immediately associate flow with ketchup as it oozes out of the bottle at a painstakingly slow pace, only to suddenly splash out in large quantities after the irritated diner customer administers a slap to the bottom of the upturned bottle. And yet, this also is flow behavior, and actually normal behavior for a liquid such as ketchup.

From these everyday experiences, where we look at flows on the centimeter, meter, and perhaps kilometer scale (lakes, oceans) we would not immediately expect liquids to behave any differently if we observed them on smaller scales – at the millimeter or even micrometer level. And yet that is exactly what happens! In this chapter we will see how many phenomena that we are so used to living with, and which we take for granted, have next to no significance for fluids in the microworld: inertia means nothing on these small scales, but viscosity rears its (hideous) head and becomes a very important player. The (seemingly) random and chaotic behavior of flows in our experience is reduced to much more well-behaved and 'smooth' (laminar) flows in the smaller domains. And diffusion, on larger

scales an almost ridiculously ineffective transport mechanism, suddenly becomes the dominant process, a strong ally or a mighty opponent, mostly depending on what you want to achieve. And, finally, surfaces become an ever more important factor to reckon with. The ratio of surface to volume increases drastically as dimensions are reduced, going from a value of $0.006\ m^{-1}$ for a cube of side length 1 km to $6\ m^{-1}$ for a cube of side length 1 m, and further to $6000\ m^{-1}$ for a cube of side length 1 mm, and finally to $6\,000\,000\ m^{-1}$ for a cube of side length 1 μm. Again, this is a 2-sided coin, where some applications greatly benefit from an increased surface to volume ratio, while in others such phenomena as adsorption become increasingly harder to deal with.

The goal of this chapter is to give you some basic insights into flow behavior at small scales and on the most important processes and phenomena that must be kept in mind when attempting to design microsystems for chemical analyses or reactions. You are of course encouraged to consult specialized books for more in-depth information (ample references are given here), but by the time you have finished this chapter you should have acquired a good primary understanding of the issues involved in designing microfluidic systems.

3.1 Fluids and Flows

Typically, a fluid can be defined as a material that deforms continually under shear stress, i.e., the application of an external force attempting to displace part of the fluid elements at a boundary layer (i.e., the surface). In other words, a fluid can flow and has no rigid three-dimensional structure. For all practical purposes, the fluids we encounter in everyday life are gases (air or its components) and liquids (water, oil, syrup, …). More complex systems consisting of several phases can also be classified as fluids (blood, suspensions, emulsions, …). Fluid behavior has been studied extensively for several centuries and a number of monographs and articles have been published on the subject (see, e.g., [1-5]). In the following, we will focus on some of the most important aspects of a fluid and its physical behavior. Since we almost exclusively deal with liquid systems in this book, the remainder of this discussion will focus on liquids only.

Three important parameters characterizing a liquid are its density, ρ, the pressure of the liquid, P, and its viscosity, η.

The density is defined as the mass, m, per unit volume, V:

$$\rho = \frac{m}{V} \tag{3.1}$$

Typical values for several fluids are listed in Tab. 3.1. We will encounter the density again in several issues, including the definition of the kinematic viscosity, discussions of surface tension and capillary forces, and when looking at the buoyancy of particles immersed or suspended in a liquid.