A Lab-on-a-Chip for Biological Fluids Analysis

G. Minas, J. S. Martins and J. H. Correia
University of Minho, Dept. of Industrial Electronics
Campus de Azurém, 4800-058 Guimarães, Portugal
e-mail: gminas@dei.uminho.pt http://www.dei.uminho.pt

Abstract – **This paper presents a microfluidic system for helping health professionals with rapid and accurate biological fluids analysis as well as for helping the patient himself at home. This microsystem consists of two wafers: a Pyrex glass wafer containing the microlaboratory (microchannels to carry chemical reagents and sample solutions) and a silicon wafer including the protein detection system (by colour analysis based on optical absorption). Albumin in urine is the first target of the microsystem, but it can be applied to other proteins. This microsystem eliminates the need of expensive readout optics and opens the road to low-cost disposable devices.**

Keywords: lab-on-a-chip, microfluidics, proteins, albumin

I. INTRODUCTION

The same basic fabrication concepts and materials which have made microelectronics successful are now being adapted to obtain low-cost, small, high-performance biomedical based systems devices, such as a microfluidic system for biomedical analysis, also referred as a laboratory on a chip (lab-on-a-chip) [1]. However, those devices have a diverse and extremely large potential use field in biomedical applications (Table 1).

Table 1: Biomedical Applications [2]

Microsystems Medical Applications	
Drug Delivery Systems • Patches • Implantable pumps • Smart pill	**Cardiology** • Pacemakers and defibrillators • Angioplasty catheters • Intravascular diagnostic
Monitoring • Point of care testing • On-line monitoring of blood gases • On-line monitoring of pressure • Dialysis control	**Analysis Systems** • Polymerase chain reaction • Genetic tests and therapy • Analysis instruments
Prosthesis/Artificial Organs • Orthopaedics • Ophthalmology • Neurology	**Minimal Invasive Surgery** • Diagnostic • Therapy

The implementation of lab-on-a-chip devices presents new and interesting technological challenges and their capabilities to chemical analysis are truly outstanding. Microscopic versions of liquid-handling devices, including pumps, valves, volume measuring tools, chemical reactors, extractors, filters, mixers and even sophisticated chromatographic techniques, can all be implemented and integrated into a chips' design, thanks to the microsystem technology which enables the fabrication of precise and small structures [3].

The microchip based technology resemble microelectronic computer chip. The microchips can be produced using photolithography and chemical etching techniques that are quite similar to those used in the manufacture of integrated circuits [4]. Microchannels are etched into the chip substrate or done by injection moulding to carry fluids: chemical reagents and sample solutions. The substrate can be glass, quartz or silicon. One example of a such microsystem is presented in Fig. 1. It includes microcomponents such as pumps, valves, flow sensors, chemical sensors and additional passive components [5]. Microsystems like this one are usually referred as Micro Total Analysis Systems (μTAS). They allow a variety of analytical chemistry methods or process control of simple mixtures, with the resolving powers of today's macro analytical systems.

The small size and portability of lab-on-a-chip devices result in a reduction of the analytical testing costs and time, and in a significant improvement in laboratory safety. Spills, explosions and other laboratory accidents that can occur with conventional sample preparation techniques are no longer a problem. Since nanoliter quantities of organic solvents and samples are used, the costs associated with buying new reagents and disposing of the used

ones are negligible [6]. Moreover, since the lab-on-a-chip rather than a chemist performs the sample preparation, untrained personnel can accurately and precisely perform a complete analysis.

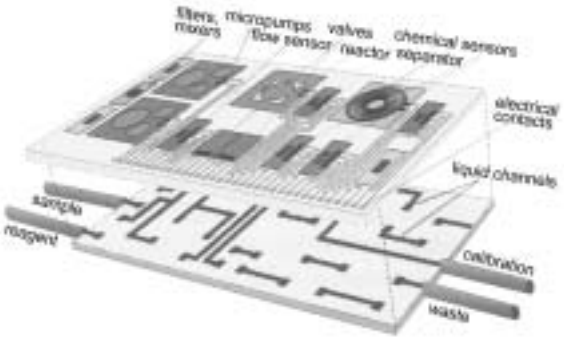

Fig. 1: Artist's impression of a μTAS [5].

In this paper a microfluidic system designed for protein concentration detection (e. g. albumin) in urine analysis is presented. The protein detection system consists in colour analysis based on optical absorption. Although this microsystem was projected for urine analysis, other biological fluids (such as blood, sweat or saliva) are potential candidates for the lab-on-a-chip.

II. MICROSYSTEM DESIGN

The microsystem itself is composed of two wafers: one containing the microlaboratory and other including the protein concentration detection system.

Microlaboratory

Fig. 2 presents a schematic top view of the microlaboratory which is fabricated in a Pyrex glass wafer. Glass was chosen for its transparency and because it is an electrical insulator [7]. Therefore, electrophoretic flow principle can be used to move fluids through the microchannels, which avoids mechanical pumps and valves. Thus, fluid movement results from electrokinetic forces derived from small voltages that are applied to specific regions in the microsystem [8].

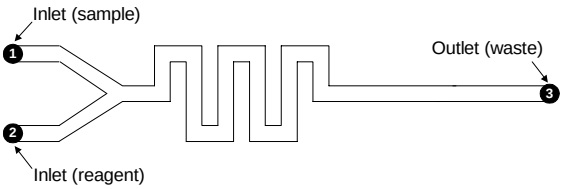

Fig. 2: Schematic top view of the microlaboratory.

The device comprises two microchannels (from inlet 1 and 2) merging into one (to outlet 3). Sample and reagent solutions are injected in the lab-on-a-chip through inlets and both solutions are mixed in the main microchannel. Low voltages are applied between electrodes placed on the silicon wafer distributed by the flow path. The mixture is analysed by a photodetector placed in the silicon wafer almost under the outlet. However, some proteins concentration detection need not only the mixing between sample and reagent but also capillary electrophoresis separation techniques (e.g. amino acid detection).

Detection System

For the colorimetric detection, a white light is used as incident light into the microchannel, where some spectral components are absorbed or reflected. The intensity of the transmitted light when measured by the photodetector can therefore give information about the proteins concentration. Once the wavelength region, which the absorption is maximum or minimum for different proteins is very narrow, the optical detector must have a high-spectral selectivity. A Fabry-Perot resonance filter is used as an effective wavelength-selecting element. It consists of two parallel mirrors with a resonance cavity in the middle, where the incident light suffers multiple reflections [9]. The equation, $2nd = \lambda q$, shows the operation principle of the Fabry-Perot filter, where n is the refractive index of the cavity medium, d the cavity length, λ the incident wavelength and q the interference order (q = 1, 2, 3,…). In short, this device acts as an optical filter that transmits certain wavelengths and reflects the others back to the light source.

III. DEVICE FABRICATION

The lab-on-a-chip is shown in Fig. 3.

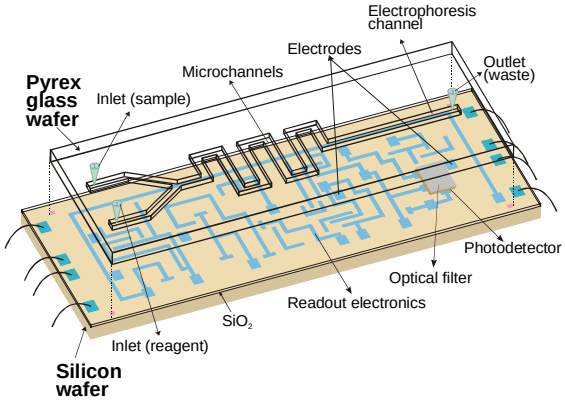

Fig. 3: Lab-on-a-chip. The top wafer shows the microchannels. The bottom wafer shows the detection system and readout electronics.

The microlaboratory consists of a Pyrex glass top wafer, where the microchannels (40 μm deep and

200 µm wide) are fabricated by wet-etching techniques. The silicon wafer has the photodetector and respective readout electronics (a light-to-frequency converter, for example). These functions are done in a CMOS (Complementary Metal Oxide Semiconductor) standard process. The Fabry-Perot layers are deposited on the silicon wafer at the very end of the fabrication sequence. Above them, a passivation layer is deposited in order to cover and protect all the previous layers (Fig. 3). At last, both wafers (the Pyrex glass and the silicon) are thermally sealed with wafer-bonding techniques.

IV. EXPERIMENTAL RESULTS

Proteins concentration detection (e. g. albumin, bilirubin, urea) in urine is the first target of the lab-on-a-chip. Proteins when bound with a specific reagent have an absorptance maximum at specific wavelengths (due to the reagent) and absorption spectra similar to those ones presented in Fig. 4 [10]. The intensity of the colour produced by the mixture is directly proportional to the protein concentration.

In order to calibrate the detection system of the lab-on-a-chip, some measurements need to be performed to find out the real transmitted wavelengths and the real relationship between protein concentration and the intensity of the transmitted light.

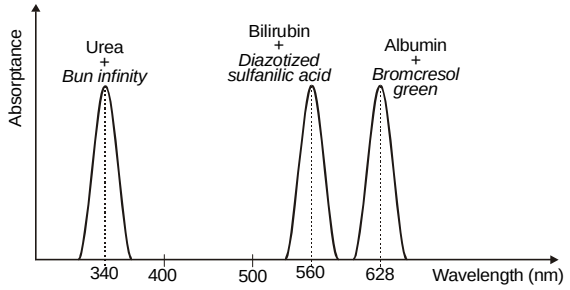

Fig. 4: Absorption spectra shape for urea, bilirubin and albumin.

Albumin Concentration Detection

Experimental results were obtained in albumin analysis, with a test kit from Sigma-Aldrich. Known protein concentrations were used in order to obtain a calibration curve and several absorption spectra. Fig. 5 shows the absorption spectra when different albumin concentrations react with bromcresol green (0.30 mmol/l, pH = 4.2). It can be seen that as far as the albumin concentration is small, smaller is the spectral peak. Measurements results were done from 50 mg/ml until 50 µg/ml albumin concentration. The normal values of albumin concentrations in human urine are between 10 and 140 µg/ml, but in human serum is higher.

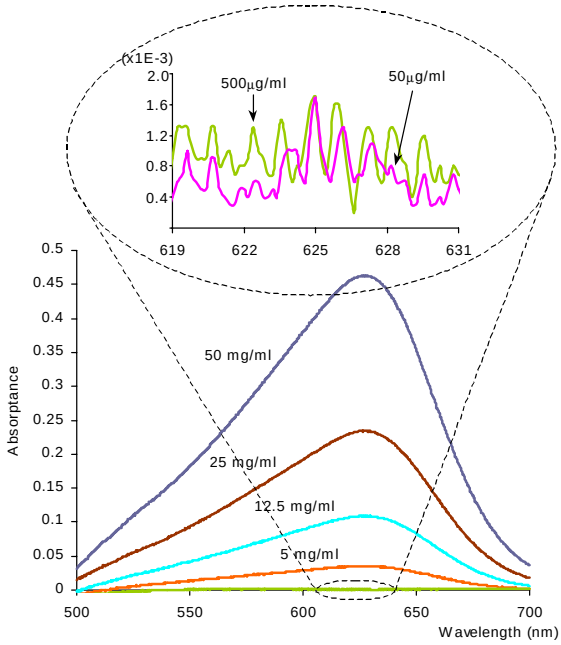

Fig. 5: Absorption spectra for different albumin concentrations, after the binding with bromcresol green.

A disadvantage of those tests is due to manual pipetting. If two different persons pipet the same concentrations solutions, the results are not the same. Furthermore, for lower concentrations, different replicas of the same concentration, result in different absorptances, even when the same person does the pipetting. These disadvantages will not occur when the lab-on-a-chip is used to do the tests, once the sample and reagent volume is computed automatically. Meanwhile, others reagents suitable for microassays are being tried (e. g. Bradford reagent, for detecting proteins total concentration).

V. CONCLUSIONS

Microtechnology allows not only the realization of microfluidic systems and optical components of reduced size, but also their assembly in stacked forms. The lab-on-a-chip presents in-situ measurements, real-time analysis, a high level of automation and a truthfulness when any untrained person perform an analysis. The microfluidic system presented here allows on-line protein concentration measurement and is highly sensitive to specific wavelengths. Therefore, such detection system is extremely suitable for application in µTAS due to its small size and high-spectral selectivity.

The lab-on-a-chip for biological fluids analysis can be a powerful tool in hospitals and operating rooms as well as in patient homes due to its rapid, accurate and sophisticated diagnostic tests for numerous critical compounds. Other applications of the lab-on-a-chip are for monitoring the air and the water quality for potential toxins and pesticides,

screening foods, and promptly identifying drugs abuse. Lab-on-a-chip devices will probably find their way into forensic, environmental and food testing laboratories in the near future. Moreover, since low quantities of hazardous chemical reagents are needed, the resultant environmental pollution starts to be no longer a problem.

VI. ACKNOWLEDGEMENTS

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