Lab-on-a-chip for Measuring Uric Acid in Biological Fluids

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SUMMARY

This paper describes a lab-on-a-chip for measuring uric acid in serum, plasma or urine. The lab-on-a-chip is composed by two dies: one is fabricated in polystyrene and contains the micro-channels and the other is fabricated through a CMOS process and contains the photodetector and readout electronics. The uric acid concentration is measured by using a mixture of 14 µl of infinityTM uric acid reagent with 0.25 µl of sample. The achieved sensitivity is 0.33 mg/dl (±0.6% of human being urine values), with a 1 mm lightpath. Using an optical absorption method, a maximum peak at wavelength $\lambda = 494$ nm, is detected.

Keywords: Lab-on-a-chip, uric acid, optical absorption.

Subject category: Biochemical sensors

INTRODUCTION

Over the past decade the miniaturization of microfluidic systems has become a highly visible and dominant trend in the physical and biological sciences [1]. Development in this area has primarily been driven by a need for rapid, on-line measurements at low concentrations, within fields such as DNA analysis, drug discovery, pharmaceutical screening, medical diagnostics, environmental analysis and chemical production. The advantages associated with shrinking analytical systems include improved efficiency with respect to sample size, response times, costs, analytical performance, integration, throughput, automation, and laboratory safety [2]. A keyword in this field is "lab-on-a-chip" or "µTAS" (micro total analysis system), where macroscopic analysis methods are being miniaturized. The small size and low consumption of these devices could make them highly portable and thus suitable for in-situ measurements tasks.

The final application for this lab-on-a-chip is the measurement of uric acid concentration in human being's urine.

Background of the Uric Acid Analysis

Uric acid is a metabolite of purines, nucleic acids and nucleoproteins. Consequently, abnormal levels may be indicative of a disorder in the metabolism of these substances. Hyperuricaemia may be observed in renal dysfunction, gout, leukemia, polycythaemia, atherosclerosis, diabetes, hypothyroidism, or in some genetic diseases. Decreased levels are present in patients with Wilson's disease. A normal adult synthesizes 26.9 to 53.8 mg/dl of uric acid in urine. In case of disease, these values can be as low as 17 mg/dl or too high up to 67 mg/dl [3].

LAB-ON-A-CHIP DESIGN

The design of the lab-on-a-chip takes into account macroscopic measurements with well-known uric acid standards performed in a 1 cm lightpath cuvette with a model UV-3101PC SHIMADZU spectrophotometer (Fig. 1). These measurements allow the following conclusions: the intensity of the color produced by the mixture is directly proportional to the uric acid concentration; the solutions present a linear behavior for concentrations as large as 30 mg/dl (for higher concentrations the sample should be diluted and re-assayed multiplying the result by the dilute factor); the absorption spectra shows a maximum peak at the wavelength $\lambda = 494$ nm, with a FWHM (Full-Width-Half-Maximum) of 90 nm.

The lab-on-a-chip is composed of two dies: one contains the microchannels to carry chemical reagents and samples, and the other contains the uric acid concentration detection system.

Polystyrene Die

Fig. 2 shows the design of the lab-on-a-chip polystyrene die. It is composed of two polystyrene

1 mm thick wafers. The first one has the holes for the injection and removing of the liquids (inlets and outlets) and the second one includes the channels. The die comprises two channels with the width of 1 mm and 6 mm long. One is needed to obtain the baseline reference and to calibrate the light source. The other allows the mixed solution analysis (reagent plus sample). The transmitted light through the mixture is measured by photodetectors placed underneath both channels in the CMOS die.

CMOS Die

The photodetectors are pn-junction photodiodes created using a p-substrate/n-diffusion junction (Fig. 3). This structure is chosen because, among the different types of photodiodes available in a standard CMOS process, it has the higher quantum efficiency in the visible range [4]. It is desirable to integrate the analog to digital conversion with the light sensors. The A/D conversion is performed by using a one-bit first-order sigma delta modulator. The input value of the converter is generated by the difference in the currents measured by the two photodiodes. Therefore, the lab-on-a-chip has a bit stream output and allows its use in small data-acquisition and control systems.



Fig. 1: Macroscopic measurements of absorbance spectra for different uric acid concentrations. From bottom to top curve: 5 mg/dl, 10 mg/dl, 15 mg/dl, 20 mg/dl, 30 mg/dl, 40 mg/dl, 60 mg/dl, 80 mg/dl and 120 mg/dl



Fig. 2: Polystyrene die



Fig. 3: Structure of a p-substrate/n-diffusion diode

LAB-ON-A-CHIP FABRICATION

Polystyrene Die

In the polystyrene die, holes and channels are drilled by using a CNC machine. Then both wafers are glued. Polystyrene is chosen due to its transparency and because it is a good insulator and easier to drill than glass. Therefore, the electrophoretic flow principle can be used to move fluids through the microchannels, which avoids mechanical pumps and valves.

CMOS Die

The photodetectors and readout electronics of the colorimetric detection system are fabricated through a double-metal, single-polysilicon, 1.6 μ m n-well CMOS standard process. The photodiodes have an active area of 500x500 μ m² each. The complete device is shown in Fig. 4. The polystyrene die is glued to the CMOS chip.



Fig. 4: Lab-on-a-chip

MEASUREMENT SETUP

Fig. 5 shows the experimental arrangement used in the measurements. A 150 W Xe lamp with a monochromator TRIAX-180 (1200 g/mm grating with a spectral dispersion of 3.6 nm/mm and a spectral resolution of 0.3 mm at 546 nm) was used as light source. Instead of this light source, white light could be used. In this case, an optical filter must be placed on the top of the photodetectors to select a wavelength range. Macroscopic measurements (Fig. 1) show that a rough band-pass optical filter will be enough, since the FWHM of the absorption spectra is 90 nm.



Fig. 5: Experimental arrangement used in the measurements

EXPERIMENTAL RESULTS

The reagent used in the measurements was the infinityTM uric acid reagent from Sigma-Aldrich. It contains approximately 0.5 mmol/l 4-aminoantipyrine, 1.75 mmol/l TBHB, >32 U/l uricase (bacillus sp.), >1300 U/l peroxidase (horseradish), buffer pH 8.0, 0.05% sodium azide as preservative [5]. This reagent reacts with a sample of urine containing uric acid in a 50:1 ratio, and produces an absorption maximum at a specific wavelength ($\lambda = 494$ nm).

Fig. 6 shows the measured transmittance response for different uric acid concentrations in urine. Measurement results are done from 5 mg/dl to 120 mg/dl, comprising the range of normal and usually abnormal values in a human being (17 to 67 mg/dl). Concentration differences less then 5 mg/dl are not taken into account, since it means a variation of less then 10% in the human being values above-mentioned. The transmittance is defined as $T = I / I_0$, where I is the measured photodiode current of each solution and I_0 the measured photodiode current of the reagent. The results obtained agree with the macroscopic measurements and the same conclusions can be achieved.

Table 1 gives some useful calculations based on the measured values. The absorption coefficient of each concentration (α) was calculated by the Beer-Lambert law:

$$I_{\lambda}(LP) = I_{\lambda}(LP = 0)e^{-\alpha_{\lambda}LP}$$

In the last column it can be seen that the minimum transmittance difference between successive measured concentrations (5 mg/dl) is 3%. However, the relative sensitivity of this device is 0.33 mg/dl (0.4%), with a 1 mm lightpath. Thus, an 8-bit analog-to-digital converter in the readout electronics is enough for this sensitivity.

The sensitivity achieved with the lab-on-a-chip is optimum when applied to the human being urine values. Therefore, the lightpath can be decreased (the sensitivity will also decrease, but the resolution of the A/D converter is kept). The sensitivity of 3.65 mg/dl (7.3% of human being urine values) can be achieved with a lightpath of 100 μ m in a 500 μ m Pyrex wafer.



Fig. 6: Measured transmittance spectra for different uric acid concentrations. From top to bottom curve: reagent, 5 mg/dl, 10 mg/dl, 15 mg/dl, 20 mg/dl, 30 mg/dl, 40 mg/dl, 60 mg/dl, 80 mg/dl and 120 mg/dl

Sols.	I (µA)	α (m ⁻¹)	<i>I/I</i> ₀	T diff
Reagent	0.177	0	1.00	
5 mg/dl	0.167	54.97	0.94	0.06
10mg/dl	0.155	117.68	0.88	0.06
15mg/dl	0.145	179.42	0.82	0.06
20mg/dl	0.141	208.64	0.79	0.03
30mg/dl	0.126	311.95	0.71	0.08
40mg/dl	0.117	378.49	0.66	0.05
60mg/dl	0.096	557.75	0.63	0.03
80mg/dl	0.085	670.15	0.48	0.15
120mg/dl	0.062	951.65	0.35	0.13

Table 1: Absorption coefficient of each measured concentration for $\lambda = 494$ nm

CONCLUSIONS

A small-size and low-cost lab-on-a-chip for real-time measuring uric acid in a urine sample is presented in this paper. The optical absorption method (with a lightpath of 1 mm) is sensitive enough for $\pm 0.6\%$ of human being urine values. The achieved sensitivity is 0.33 mg/dl. This detection system avoids the need of expensive readout optics and opens the door to low-cost disposable devices. Moreover, tests using ambient light have been done to avoid the use of a known source of light. Although this microsystem is presented for urine analysis, other biological fluids (such as serum, sweat, saliva or cerebrospinal fluid) are potential candidates for the lab-on-a-chip.

There are other potential applications for the lab-on-a-chip, namely: monitoring of air and water

quality (looking for toxins and pesticides, etc.) and fast identification of 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 is negligible.

ACKNOWLEDGMENTS

This work is supported by Foundation of Science and Technology, Portugal, FCT, SFRH/BD/1281/ 2000 and FCT, POCTI/33747/ESE/1999 and FEDER.

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