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A SU-8 fluidic microsystem for biological fluids analysis

J.C. Ribeiro [a,*], G. Minas [a], P. Turmezei [b], R.F. Wolffenbuttel [b], J.H. Correia [a]

[a] *University of Minho, Department of Industrial Electronics, Campus de Azurém, 4800-058 Guimarães, Portugal*
[b] *Delft University of Technology, Faculty of ITS, Department of Microelectronics, Mekelweg 4, 2628 CD Delft, The Netherlands*

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Abstract

This paper describes a fluidic microsystem fabricated in SU-8 epoxy photoresist suitable for on-chip liquid handling and mixing. The application is the concentration measurement of biomolecules in biological fluids, such as urine, blood, serum, plasma or cerebrospinal fluid.

In general, fluidic microsystems on glass are fabricated using HF acid etching techniques, which requires careful handling, or using micromilling, which gives high-roughness surface. The SU-8 fabrication process is low-cost, biocompatible and UV lithography semiconductor compatible. It requires neither complex techniques nor expensive masks and is suitable for batch production. Moreover, SU-8-based processing enables the fabrication of deep microchannels with very low sidewall roughness, which is suitable, when it is used an optical absorption measurement method. The high light of this paper is the implementation of a fluidic microsystem for biological fluids analysis with SU-8 photoresist. The fluidic microsystem performance is successfully demonstrated in the spectrophotometric measurement of total protein concentration in urine, by optical absorption.

Keywords: SU-8 techniques; Fluidic microsystem; Biomolecules analysis

1. Introduction

Disease prevention and treatment is often based on the measurement of several chemical parameters in biological fluids. Optical absorption measurement at a specific wavelength is a very convenient and common analytical technique for the selective detection of the concentration of biomolecules in routine tests analysis [1]. However, in most cases, the samples of biological fluids for those tests analysis need to be sent to a central laboratory for analysis and the test results become available after several hours and sometimes days. The need for a rapid measurement at low concentrations has led to miniaturization of fluidic analysis systems with the fluidic, the detection and the readout electronics integrated in a single microsystem. These features are essential within fields such as DNA analysis, drug discovery, pharmaceutical screening, medical diagnostics, environment analysis and chemical production [2]. The advantages associated with reducing the dimensions of clinical analyses systems include improved efficiency with respect to sample size, analytical performance, automation, integration, laboratory safety, response times and costs [3].

An essential requirement required for any practical fully integrated chemical analysis systems in a single microsystem is the ability to mix two or more fluids thoroughly and efficiently (i.e. in a reasonable amount of time). These microscale fluidic systems have distinctive properties as a result of their small dimensions. First, liquid flow is generally laminar, not turbulent. Second, diffusion is the only practical process for mixing fluids in narrow channels. Third, particles can be separated by diffusion according to their size [4].

2. SU-8 versus HF etching and micromilling techniques

Micromilling or HF acid etching techniques are usually used in fluidic microsystems fabrication. Micromilling

* Corresponding author. Tel.: +351 253510190; fax: +351 253510189.
E-mail address: jcaribeiro@dei.uminho.pt (J.C. Ribeiro).

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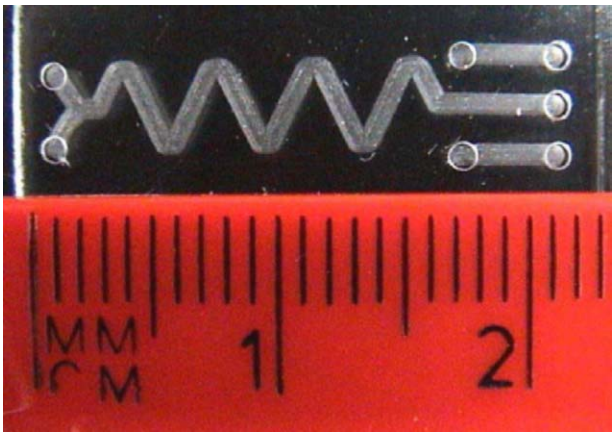

Fig. 1. Fluidic microsystem fabricated by micromilling techniques. The channels are 1 mm width and 500 μm depth [5].

techniques were used to fabricate a microfluidic microsystem in glass, as is shown in Fig. 1 [5]. It is a simple process and uses standard drilling equipment. However, this technique is not suitable for batch production, it suffers from high-roughness surface and sometimes it can crack the glass. A HF etching device was fabricated by Berthold et al. [6] and it is presented in Fig. 2. The HF acid etching technique is complex, expensive and can be very dangerous if mishandled. It needs an enclosed area, equipment and materials for its exclusive use. Both these prototypes were tested with success for analysing biomolecules in biological fluids [5–7].

The SU-8 is an epoxy-based resin photoresist. It has excellent mechanical properties, it allows etching of deep channels up to 1 mm. It is suitable for fabrication of microstructures with a high aspect ratio (typically in order 1:20 to 1:25, but can be about 1:50) and smooth vertical sidewalls. These features are appropriated for optical absorption measurement, once it requires optical paths higher than 500 μm [8]. The SU-8 process does not require an enclosed area and the equipment (such as a hot spin coating and a standard lithograph) is not expensive, compared with HF acid etching. Moreover, its equipment can also be used in other applications. Additionally, the SU-8 process exhibits excellent chemical resistance, very good biocompatibility, UV lithography semiconductor compatible and does not require expensive masks [9].

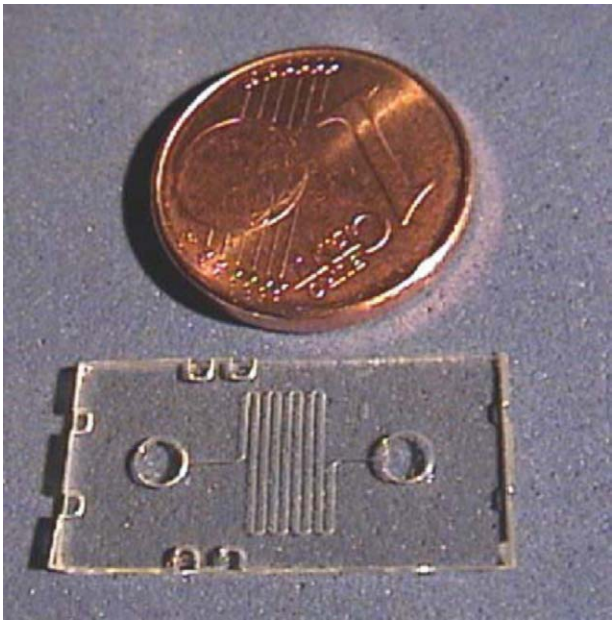

Fig. 2. Fluidic microsystem fabricated by HF etching techniques [6].

3. Model and design

The fluidic mixer is an integrated part of the biological microsystem. It is designed for enabling a mixing process driven by diffusion, which allows an easy-to-fabricate and low-cost mixer. A computer simulation, using Ansys Flotran with the Flotran CFD option, has been carried out to analyse the details of the flow and the diffusion in the mixer in order to derive the appropriate design criteria for the layout [4]. The evaluation of the mixing process was carried out using the fast-reaction of 'Microprotein-PR' reagent with urine samples.

The mixing process for total protein concentration of 7.5 and 50 mg/dl with a flow rate of 2.3 mm s^{-1} is shown in Fig. 3. The normal range concentrations in human's urine are up to 15 mg/dl. At inlet 1, 9.5 μl of reagent enters the mixer and at inlet 2, 0.19 μl of urine sample.

The liquids were introduced at the inlets and fed through the mixer to the outlet. The liquid pressure in the outlet was kept at zero. Driven by the liquid pressure, liquids pass through the U-shaped intersection. Incompletely mixed zones are seen in the mixer (Fig. 3), but near the fifth U-turn the mixing is complete and homogeneous, the viscosity value reaches 0.9E − 3. This is the theoretical and experimental viscosity value (measured in a viscosimeter) of the mixture when both liquids are mixed. This basic geometric layout fulfils the requirements for total protein analysis in a human's urine, since the mixing between the sample and the reagent is completed before reaching the detection chamber.

The prototype structure of the integrated fluidic die is illustrated in Fig. 4. The liquids enter and exit the device through inlets and outlet holes drilled in the top glass wafer. The top glass wafer was glued on the SU-8 microchannels. A special glue is used for the adhesive bonding of both wafers. After the liquids enter trough, the inlets they flow parallel to the glass substrate through the main flow channel.

4. Fabrication process

The channels are fabricated using a layer of photoresist SU-8 deposited on the glass substrate, which gives the required rectangular vertical profile of the channels. A negative mask is obtained with a regular transparency foil and is shown in Fig. 5.

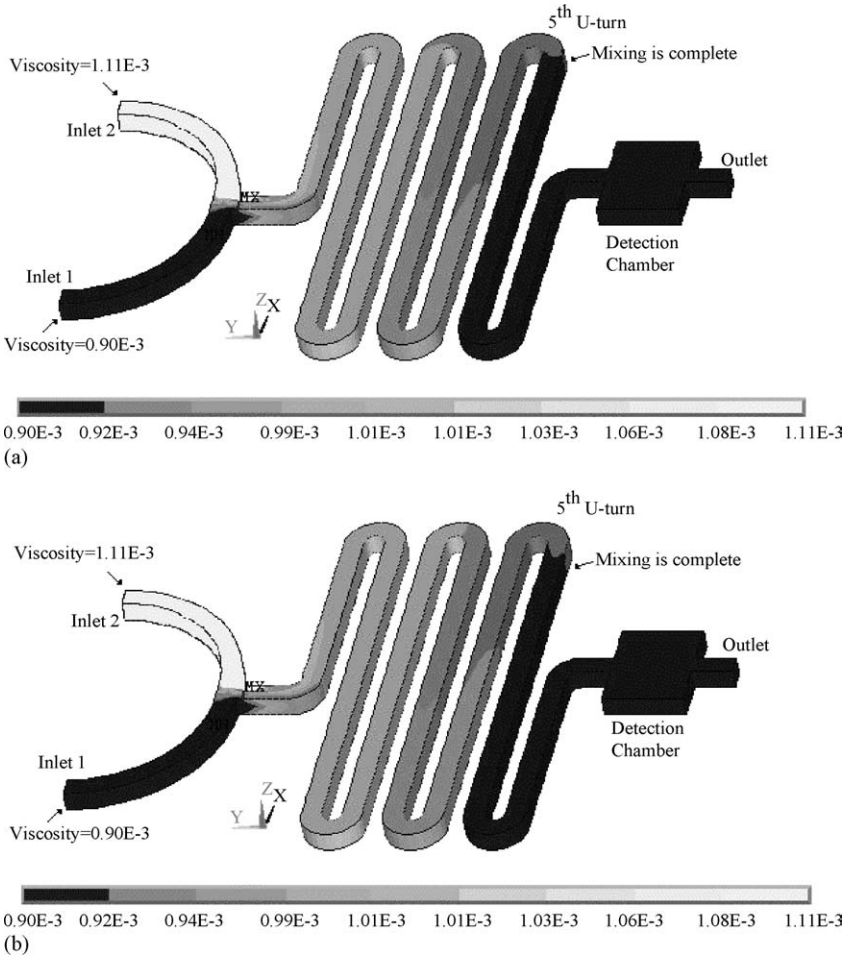

Fig. 3. Simulation of the flow for diffusion mixing for (a) 7.5 and (b) 50 mg/dl of total protein concentration in urine. The lower graphic bar shows the viscosity profile of the model. Channels have width and depth of 500 μm. The main channel is 70 mm long. The detection chamber is 2 mm × 2 mm.

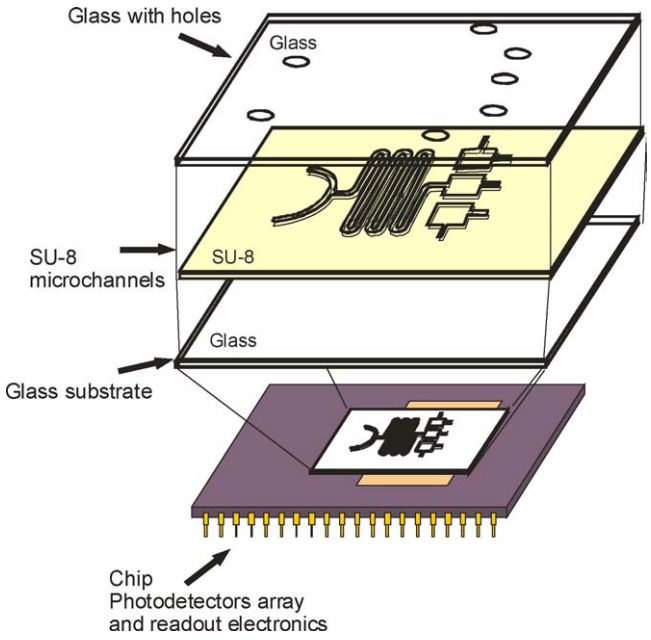

Fig. 4. A drawing of the constituent prototype pieces integrated fluidic die.

The photoresist chosen was SU-8 100. The SU-8 was first spun on a glass substrate at 700 rpm for 100 s. The pre-expose bake was performed by soft bake at 90 °C for 3000 s. The second spin was done at 700 rpm for 100 s and soft bake for 300 s at 50 °C. After, it was ramped up to 90 °C, for 5400 s, and ramped down. The exposure was for 900 s on an Electronic Vision EV-420 mask and bonding aligner. Post-exposure bake for 300 s at 50 °C. Ramp up to 90 °C for 1200 s, and ramp down. The development took 2700 s in the SU-8 developer.

The fluidic microsystem fabricated using SU-8 is shown in Fig. 6. The channels are 600 μm deep and 500 μm width.

The CMOS compatible photodetectors and readout electronics of the colorimetric detection system were fabricated through a double metal, single polysilicon, 1.6 μm *n*-well CMOS standard [5].

5. Experimental results

The reagent used in the measurement of the total protein in urine was the Microprotein-PR, procedure no. 611 [1]. It contains the red pirogalol-molibdato, which reacts with the

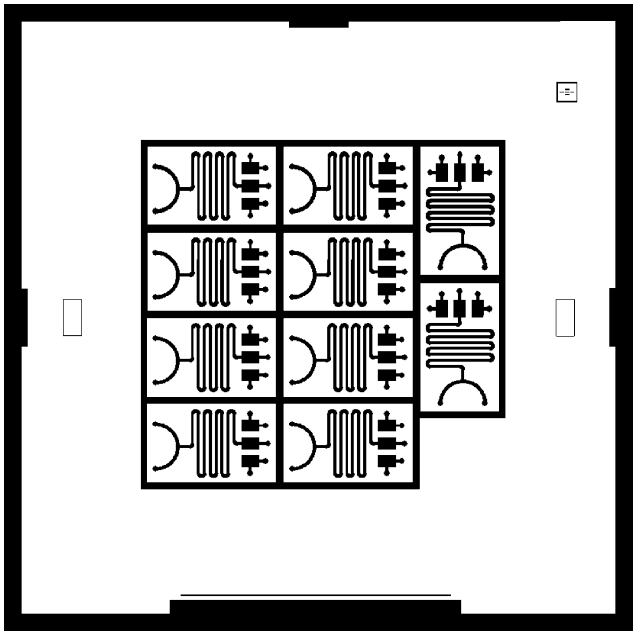

Fig. 5. The SU-8 negative mask used for the fabrication of the fluidic microsystem.

amino acids groups of the protein molecules. The procedure is simple, not corrosive and it is specific for low concentrations measurements in total protein (particularly in urine). The Microprotein-PR reagent reacts with a sample of urine containing proteins in a 50:1 ratio, and produces an absorption maximum at a specific wavelength ($\lambda = 600$ nm).

Fig. 7 presents the measured transmittance response for different total protein concentration in urine, obtained from a photodiode placed underneath of the fluidic microsystem. The measured results are done from 3 to 100 mg/dl, comprising the normal and the abnormal values in a human being. The measurements are done for 10 different assays and also for 10 different measurements in the same assay, for each concentration. The reproducibility and repetitiveness values obtained are less than 9.0%. The transmittance is defined as $T = I/I_0$, where I is the measured photodiode current for each solution and I_0 the measured photodiode current of the reagent. The transmittance, at $\lambda = 600$ nm, as a function of the different total protein concentration is shown in Fig. 8. The experimental results show that: (1) the intensity of the colour produced by the mixture is directly proportional to the total protein concentration; (2) the solutions concentration present a linear behaviour, it can be seen by the calibration curve of Fig. 8; (3) the transmittance spectra shows a maximum absorbance at the wavelength $\lambda = 600$ nm, with a full-width-half-maximum (FWHM) of 90 nm; (4) an increase of 1 mg/dl in the protein concentration corresponds to a change of 0.00025 in the transmittance value.

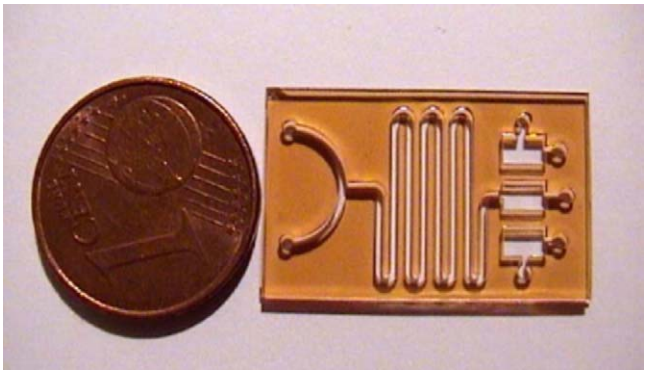

Fig. 6. A photograph of the fabricated SU-8 fluidic microsystem.

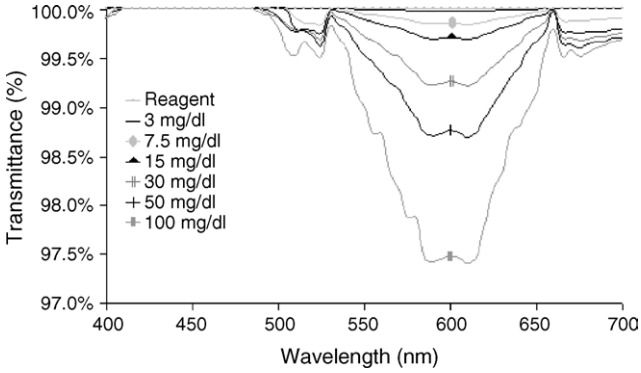

Fig. 7. Measured transmittance spectra for different total protein concentrations.

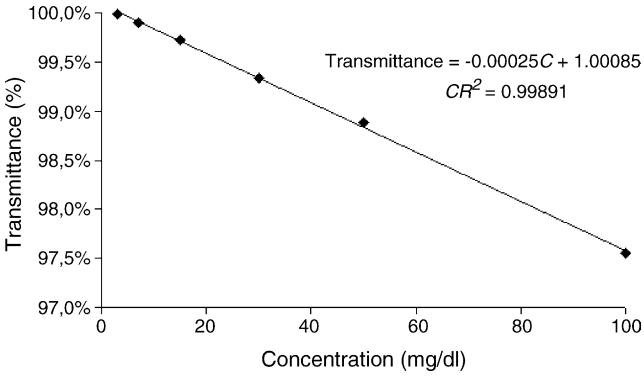

Fig. 8. The transmittance at $\lambda = 600$ nm as a function of the different total protein concentration.

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

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

Table 1
Absorption coefficient of each measured concentration for $\lambda = 600$ nm and with LP = 500 μm

Solutions (mg/dl)	T (%)	α (m^{-1})	T_{dif} (%)
Reagent	100.00		
3	99.98	0.27	0.02
7.5	99.89	2.05	0.09
15	99.72	5.66	0.17
30	99.33	13.19	0.39
50	98.88	22.40	0.45
100	97.55	45.82	1.33

where LP is the light path (500 μm in that case). In the fourth column it can be seen that the minimum transmittance difference (T_{dif}) between successive measured concentrations (3 mg/dl) is 2%. This relative sensitivity is suitable for human being urine values [10].

6. Conclusions

A fluidic microsystem fabricated in SU-8 and suitable for measuring the concentration of biomolecules in biological fluids is presented. The sensitivity achieved in the measurements of total protein concentration in urine is appropriate for human being values. The SU-8 fabrication process is a low-cost process, biocompatible, UV lithography semiconductor compatible and does not require expensive masks. SU-8 techniques for fabrication of fluidic microsystems compete favourably with other conventional techniques. This fluidic microsystem avoids the need of expensive readout optics and opens the door to low-cost disposable devices.

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Biographies

José Carlos Ribeiro graduated in electrical engineering at University of Minho, Portugal in 2003. Since March 2004 he has been a lecturer in the Electrical Department of Polytechnic Institute of Bragança, Portugal and he is involved in biomedical microdevices research.

Graça Minas graduated in industrial electronics engineering in 1994 and obtained her MSc degree in 1998 both titles at University of Minho, Portugal. Since 1995 she has been a lecturer in Department of Industrial Electronics, University of Minho, Portugal and she is involved in biomedical microdevices research.

Peter Turmezei was born in Budapest, Hungary, in 1976. In 1995, he started his higher education at the Kando Kalman Polytechnic (KKP), Hungary, where he received his BSc degree in mechanical engineering. Meanwhile, he followed a joint program of KKP and Nottingham Trent University (NTU) and received the BSc degree of NTU in integrated engineering in 1998. He continued his studies at the Technical University of Budapest, Hungary, and received his MSc degree in integrated engineering in 2000. Currently is a PhD student at the Delft University of Technology, The Netherlands, where he works on the design of an integrated particle shape detector.

Reinoud F. Wolffenbuttel received the MSc and PhD degrees from the Delft University of Technology, Delft, The Netherlands, in 1984 and 1988, respectively. Between 1986 and 1993, he was an assistant professor, and since 1993, an associate professor, at the Laboratory of Electronic Instrumentation of the Delft University of Technology, where he is involved in instrumentation and measurement in general and on-chip integration of microelectronic circuits and silicon sensor, fabrication compatibility issues, and micromachining in silicon and microsystems, in particular. He was a visitor at the University of Michigan, Ann Arbor, in 1992, 1999 and 2001, at Tohoku University, Sendai, Japan, in 1995, and at EPFL, Lausanne, Switzerland, in 1997. Dr. Wolffenbuttel was the recipient of a 1997 NOW pioneer award. He served as General Chairman of the Dutch National Sensor Conference in 1996 and Eurosensors in 1999.

Jose Higino Correia graduated in physical engineering from University of Coimbra, Portugal in 1990. He obtained in 1999 a PhD degree at the Laboratory for Electronic Instrumentation, Delft University of Technology, working in the field of microsystems for optical spectral analysis. Presently, he is an associate professor in Department of Industrial Electronics, University of Minho, Portugal. He was the general-chairman of Eurosensors 2003, Guimaraes, Portugal. His professional interests are in micromachining and microfabrication technology for mixed-mode systems, solid-state integrated sensors, microactuators and microsystems.