

SU-8 microfluidic mixer for use in lab-on-a-chip devices for biological fluids analyses

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Abstract- *This paper describes an easy-to-fabricate and low-cost SU-8 microfluidic mixer suitable for enabling a mixing process based on diffusion. It is developed to be an integrated part of a lab-on-a-chip for measuring the concentration of four biomolecules in urine samples, by optical absorption. The design of the microfluidic system is based on computational fluid dynamics techniques. Mixing and reaction of the components of the process must be simulated by solving the flow and mass transport equation. A good design must guarantee the mixing of the reactants to assure an uniform mixture at the detection zone. The resulting design and the experimental results are supported by numerical simulations, which allow a reliable quantitative analysis of the concentrations after the mixing process. The reduced size, weight and the simultaneous measurement of more than one biomolecule concentration will improve the performance of biological fluids analyses in clinical laboratories and consequently the quality of the medical diagnostic.*

I. INTRODUCTION

The healthcare sector is nowadays one of the most dynamic and where the novelty is a strategic and operational imperative. The possibility of increase the quantity and quality of clinical analysis, performed with instantaneous results and outside the clinical laboratories, contributes to a better quality in the health care services and also a better efficiency in the clinical and administrative processes [1]. This possibility can be achieved with the presented lab-on-a-chip for spectrophotometric analysis of biological fluids. It promises significant improvement over conventional analytical instruments in terms of speed, cost, sample and reagent consumption, contamination, efficiency and automation.

For prevention, diagnostic and disease treatment patients are often subject to biochemical analysis of their biological fluids, such as whole blood, serum, plasma, urine, cerebrospinal fluid, etc. However, these analyses are, usually, carried out in clinical analysis laboratories and the results become available after several hours or sometimes days. As a consequence a reliable diagnosis cannot be performed within the consultation time. Also, mistakes in the logistics, such as lost samples and mislabeling, may further delay diagnosis [2]. In a state-of-the-art laboratory there are automated equipment that can reduce those errors, but they use high sample and reagent volumes (about 1 ml), which makes the analysis systems expensive and does not contribute to patient comfort. Outside the laboratory environment, and commercially available, there are the reagent strips. However, they are available for a limited set of

biomolecules to be analyzed, usually 10 to 12, and the color readout is merely qualitative. The need for rapid measurements with low sample volumes has led to the development of lab-on-a-chips with the fluidic, detection and readout systems integrated in a single chip [3].

II. BACKGROUND OF SPECTROPHOTOMETRIC ANALYSES AND MICROFLUIDIC MIXING

The spectrophotometric analysis (the study of the interaction of electromagnetic radiation with (bio)chemical compounds) is a very convenient and often used analytical technique in clinical laboratories for routine tests analyses, especially the one based on colorimetric detection. It allows the selective detection of the concentration of biomolecules in biological fluids samples [4]. The measurement is based on colorimetric detection by the optical absorption in a part of the visible spectrum defined by the biomolecules present in the fluid sample. These biomolecules have an absorption maximum at a specific wavelength. The absorbance value at this wavelength is directly proportional to the concentration of those biomolecules in the samples. However, many of the analytes (an analyte is the substance being analyzed) of interest for clinical analysis do not have chromophores that absorb light in a useful part of the visible range. Specific chemical reactions are available (reagents) to transform these analytes into colored products that do have adequate absorbance [4]. In addition, the biomolecules concentration is measured by using a mixture of a reagent with an analyte sample.

One of the most important procedures for any practical fully integrated biochemical analysis systems is the ability to mix two or more fluids thorough and efficiently, i.e., in a reasonable amount of time. The micrometer scale dimensions of such microfluidic mixers induce laminar flow, due to the low volumetric flow rate (Reynolds numbers less than 1), meaning that diffusion is practically the only process for mixing fluids [5]. An easy-to-fabricate and low-cost SU-8 microfluidic mixer suitable for enabling a mixing process driven by diffusion is reported. It is an integrated part of a lab-on-a-chip for biological fluids analysis. Due to its low-cost, the microfluidic mixer can be a disposable part of the device avoiding, in addition, the costs associated with cleaning of the microchannels.

III. MICROFLUIDIC SYSTEM DESIGN

A. Fluids Characteristic

The application of the reported lab-on-a-chip is the simultaneous measurement of the concentration of calcium, chloride, creatinine and glucose in urine samples, by colorimetric detection. The evaluation of the mixing process was carried out using the fast-reaction of a specific reagent with a urine sample. Each reagent is selective to a single biomolecule and when both bonds the colored product is produced. The intensity of that color determines and it is directly proportional to the biomolecule concentration.

The fluid properties of the reagents and the samples incite that they have good characteristics for being mixed, in a reasonable amount of time, using only diffusion [4]. Therefore, a simple geometry (Fig. 1) was implemented.

B. Mixing Process Simulation

The microfluidic mixer is composed of a dispensing zone (inlets 1 to 5), a fluid transportation zone (the main microchannels) and a detection zone (dashed square in Fig. 1). In the dispensing zone the sample and the reactants are supplied to the system. In the transport zone reactants and the sample are mixed and transported to the detection zone. In the detection zone the reaction is finished, a complete and homogeneous mixing is obtained and the optical detection is performed. The microfluidic mixer has just one inlet for the urine sample (inlet 1 in Fig. 1) and four inlets for the specific reagents (inlets 2, 3, 4 and 5 in Fig. 1). The urine sample reacts with the reagents and the detection device measures the absorbance of the reaction products. In addition, the urine sample is mixed with the respective reactants (including the enzymes that catalyze the biological reactions). Initially, the mixing channels are filled with a buffer solution. Then, the sample and the reactant are supplied to the system getting in contact at the Y junctions. Mixing and reaction take place along the transportation channel and before the detection zone.

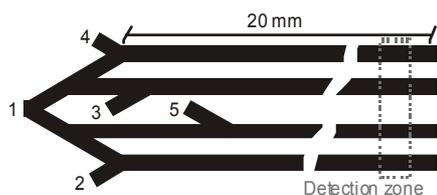


Fig. 1. Microfluidic mixer layout. Inlet 1 is for urine. Inlets 2, 3, 4, and 5 are for the reagents of glucose, creatinine, chloride and calcium, respectively. All channels are 400 μm wide.

Due to the slightly different diffusion coefficient and kinetic constants of the four biomolecules (presented in Table I), the main channel has different lengths, performed by displacement the inlets of the four reagents (x -axis). However, for a better understanding of the details of the fluid flow in the microfluidic mixer device and the mixing behavior, fluid dynamic simulations were performed. Using FEM CAD (Finite Element Method Computer Aided Design) software, pressure driven flow is studied. Numerical methods are used to simulate

the reaction and mixture in the system. Velocity-pressure formulation of the Navier-Stokes equations is solved by a finite difference method. Mass transport equation is solved by a second order finite difference method. For enzymatic reactions, biochemical reaction kinetics is considered. The mixing process for a glucose concentration of 50 mg/dl and a flow rate of 0.5 mm s^{-1} is shown in Fig. 2. Urine sample with glucose is introduced at the upper inlet and the reagent at the bottom inlet. The liquid volume in the main channel is 4 μl . The figure shows the normalized values of the contour plot of the mass fraction of 50 mg/dl of glucose in urine. The analyte diffuse into the reagent in the downstream microchannels before the mixture reach the detection zone. Due to the low fluidic flow rate (0.5 mm s^{-1}) the fluid flow is laminar with low Reynolds number (upper bound on the Reynolds number is 1.13). Incompletely mixed zones are seen, near the Y junction, in the microfluidic mixer. However, after 12 mm, from the Y, the mixing is complete and homogeneous. In addition, a complete and homogeneous mixing of the reagent with the sample is obtained before the mixture reaches the detection zone. This characteristic allows an easy-to-fabricate microfluidic mixer suitable for SU-8 techniques.

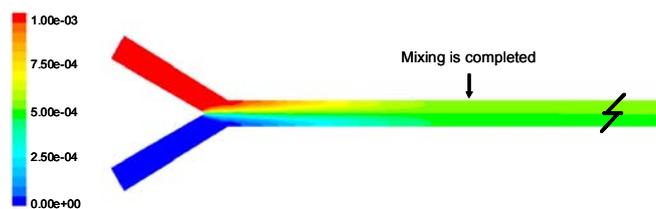


Fig. 2. Contour plot of the mass fraction of glucose with a diffusion coefficient of $D = 0.49 \times 10^{-6} \text{ cm}^2/\text{s}$, for a glucose concentration of 50 mg/dl in urine. The numerical grid contains 21077 quadrilateral cells.

Similar simulations were performed for the other three biomolecules reported (calcium, chloride and creatinine), using the diffusion coefficients and kinetic constants presented in Table I. The simulation results are very similar, once the properties of the reagents are also very similar. The main difference is the distance from the Y junction needed for the completed mixing. However, this distance changes from 10 mm to 18 mm.

TABLE I
DIFFUSION COEFFICIENTS AND KINETIC CONSTANTS OF THE FOUR BIOMOLECULES IN THE SPECIFIC REAGENT

Biomolecule	Diffusion coefficient	Kinetic constant
Glucose	$0.49 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$	$0.1789 \text{ k}/(\text{g/L})^{n-1} \text{ min}^{-1}$
Calcium	$1.65 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$	Instantaneous reaction (order of the reaction $n = 0$)
Chloride	$0.078 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$	$0.0080 \text{ k}/(\text{mmol/L})^{n-1} \text{ min}^{-1}$
Creatinine	$1.00 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$	$0.002 \text{ k}/(\text{g/L})^{n-1} \text{ min}^{-1}$

Note: The diffusion coefficients were obtained from the reagents datasheets. The kinetic constants were obtained experimentally by three methods (initial velocities, differential and integral). The presented value is the one that has a slower standard deviation.

C. Lab-on-a-chip Structure

Fig. 3 shows a drawing of the lab-on-a-chip structure. The CMOS (Complementary Metal Oxide Semiconductor) chip, under the microfluidic mixer, for reading the intensity of the produced color comprises photodetectors and readout electronics. The photodetectors are CMOS photodiodes optimized for having the best quantum efficiency in the wavelengths for which each mixture has its absorption maximum [6]. The readout electronics contains an analog multiplexer for selecting the channel to be measured and a light-to-frequency converter to transform the photocurrent into a digital signal for interfacing with a computer. The CMOS chip can be used for more than one analysis. While the microfluidic mixer, if fabricated in a low-cost process (as the one described here), can be a disposable die, which avoids the costs associated with the cleaning of the channels.

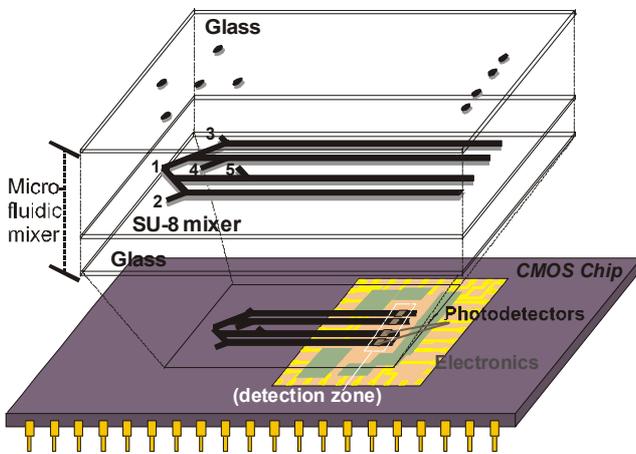


Fig. 3. Drawing of the lab-on-a-chip structure.

IV. MICROFLUIDIC SYSTEM FABRICATION

The microchannels are fabricated using a layer of photoresist SU-8 deposited on a glass substrate, which gives the required rectangular vertical profile of the microchannels. The SU-8 photoresist is an epoxy-based material that offers good properties, such as high mechanical strength, good adhesion on many different substrate materials and biocompatibility. The SU-8 based fabrication is a low-cost process, UV (between 350 nm and 400 nm) lithography semiconductor compatible and does not require expensive masks. It can be processed with a spincoating and an UV maskaligner. In addition, the microfluidic system can be a disposable die, which minimize the cost associated with cleaning of the microchannels and avoids the contamination between analyses. Moreover, SU-8-based processing enables the fabrication of deep microchannels with very low sidewall roughness which is suitable for optical absorption measurement [7].

The negative mask to be used for patterning the microchannels structure over the glass substrate is fabricated from a regular transparency foil (like the one used in printed

circuit boards). The SU-8 photoresist chosen is the SU-8-2150, which has a high viscosity and it is the most appropriate for the required channels depth. The following paragraph describes the SU-8 processing steps.

After cleaning and drying the glass substrate, the microchannel fabrication starts with the spin of a 1.25 ml/cm^2 of SU-8-2150 in this substrate. This process requires two steps: first, ramp up of the spin rotation to 500 rpm during 5 s and keep that velocity during more 10 s; second, ramp up again the spin rotation to 1000 rpm during 3 s and keep it during 30 s. Then, a soft bake process is needed for evaporation of the solvent and for the hardening of the SU-8. The soft bake is performed at 65°C during 420 s and at 95°C during 3600 s. The initial temperature allows a slower evaporation, which results in a better coating and, mainly and very important, without grooves. Next, the UV (between 350 nm and 400 nm) exposition takes place. An excessive dose of light, lower than 350 nm, results in an excessive absorption at the surface of the film. The effect can be a pyramidal shape of the microchannels. To avoid this effect, a commercially available glass optical filter was used to cut of the wavelengths lower than 350 nm. The used exposition energy was 700 mJ/cm^2 . The post-exposure bake is performed at 65°C during 60 s. Next, the temperature is ramped up to 95°C , then kept constant for 900 s and ramped down again. Fig. 4a shows a cross-section of the device after these steps. The microchannels fabrication ends with the development, during 2400 s, in the SU-8 developer (an agitation bath of 1-methoxy-2-propanol acetate). The unexposed resist is dissolved and the microchannels are formed. The structure is then cleaned with isopropanol and deionized water and dried with nitrogen. Fig. 4b shows a cross-section of the device after these steps.

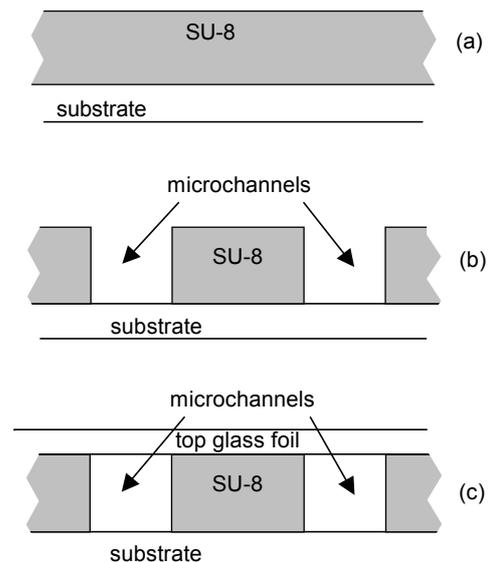


Fig. 4. Fabrication sequence of the 1st prototype microfluidic mixer: (a) spin coating, soft bake, UV exposure and post-exposure bake of the SU-8; (b) development of the SU-8; (c) placement of the top glass foil.

For the fabrication of the top glass foil, firstly, it was spun above it a very thin layer of SU-8. Next, the foil was subject to the same soft bake at 95 °C, as the previous structure for 3600 s. After, the holes were drilled using a computer numerically controlled machine. Special care must be taken with the drill tool: its velocity must be slow and the glass foil must be wet with oil in order to avoid its damage by excessive temperature. The two glass foils are then bonded together by aligning and pressing against each other with a hard bake temperature of 150 °C. Fig. 4c shows a cross-section of the device after these steps and Fig. 5 shows a picture of the 1st prototype microfluidic mixer structure.

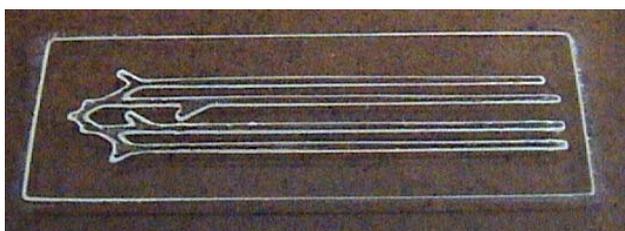


Fig. 5. Picture of the 1st prototype microfluidic mixer.

V. EXPERIMENTAL RESULTS

The experimental arrangement used in the measurements of the color produced by the mixtures comprises a 250 W quartz tungsten halogen lamp with a ORIEL Cornerstone 130TM monochromator (1200 l/mm grating with a spectral dispersion of 6.6 nm/mm and a spectral resolution of 0.5 nm at 350 nm) and an optical fiber to guide the light to the microfluidic mixer, which were used as light source. A Keithley 487 picoammeter (full-scale range from 10 fA to 2 mA and a resolution of 5-1/2 digit) is also used for measuring the photodiode current.

Proper operation of the lab-on-a-chip is confirmed using a set of experiments that involve the quantitative measurement of glucose, calcium, chloride and creatinine in urine. All the measurements concentrations comprise the range of normal and abnormal values in a human being. The reagents and the standards used in the measurements of the concentration of those four biomolecules were supplied by BIOLABO Reagents. The procedures are simple and not corrosive. All of them form a colored complex and the absorbance of that complex is proportional to the concentration of the biomolecule in the specimen and has its absorption maximum at a specific wavelength.

A detail of the glucose analysis method is described next. The method is based in the Trinder Method [8, 9]. The glucose is oxidized to gluconic acid and hydrogen peroxide which in conjunction with POD (Peroxidase), reacts with chloro-4-phenol and PAP (4-Amino-antipyrine) to form a red quinoneimine. The absorbance of the colored complex is proportional to the concentration of glucose in the specimen and has its absorption maximum at 508 nm. A detailed description of the calcium, chloride and creatinine analysis

methods can be found in [10-12]. Studies of the influence of some main substances found in urine on the determination of glucose were carried out. For example, direct bilirubin, ascorbic acid up to 10 mg/dl, total bilirubin above 20 mg/dl, did not interfere significantly with the analytical procedure. DS Young [13] has published a comprehensive list of drugs and substances that may interfere with this assay, as well as with the other three assays.

Fig. 6 shows the transmittance spectra for different glucose concentration in urine. The transmittance is defined as $T = I/I_0$, where I is the measured photodiode current for each mixture and I_0 the measured photodiode current of the empty channel (baseline reference). The optical lightpath is 500 μm (the depth of the microchannels). The calibration curve at $\lambda = 508 \text{ nm}$ that allows calculating the resultant fit parameter of Table II is found in Fig. 7. With a correlation coefficient of 0.9957, the fit can be considered linear.

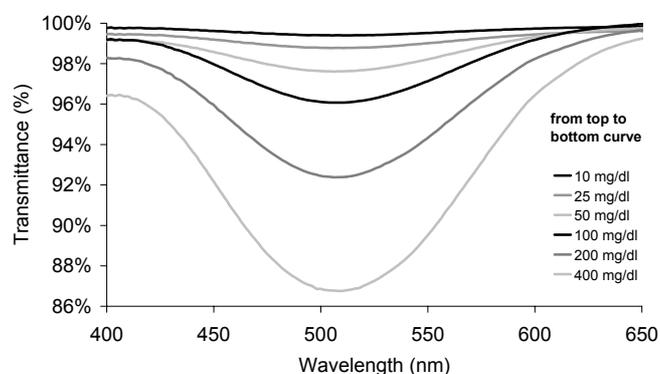


Fig. 6. Measured transmittance spectra for different glucose concentration in urine, with a photodiode placed underneath the microfluidic mixer (optical lightpath LP = 500 μm).

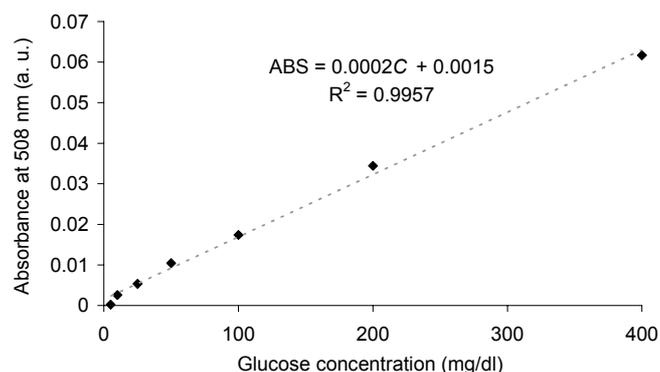


Fig. 7. Calibration curve for different glucose concentration in urine.

TABLE II
RESULTING FIT PARAMETERS OF THE CALIBRATION CURVE FOR DIFFERENT GLUCOSE CONCENTRATION IN URINE

Linear concentration range (mg/dl)	Slope (a. u.) dl/mg	Intercept (a. u.)	Correlation coefficient R^2
0 - 400	$(2.0 \pm 0.05) \times 10^{-4}$	$(1.5 \pm 0.8) \times 10^{-3}$	0.9957

The experimental results show that: (1) the intensity of the color produced by the mixture is directly proportional to the glucose concentration; (2) the solutions concentration present a linear behavior (Fig. 7 and Table II); (3) the transmittance spectra shows a maximum absorbance at the wavelength $\lambda = 508$ nm, with a full-width-half-maximum of 95 nm; (4) an increase of 1 mg/dl in the glucose concentration corresponds to a change of 0.0002 in the transmittance value; (5) and the reproducibility and repetitiveness mean coefficient variation values obtained for 10 different assays and 10 different measurements on the same assay are less than 10%.

These measurements confirm the direct proportionality between intensity of the color produced by the mixture and the biomolecule concentration. Moreover, they agree with macroscopic measurements performed with well-known standards and using state-of-the-art laboratory equipment, which have been reported in literature [14].

Similar experiments were made for calcium, chloride and creatinine. The results are shown in Figs. 8 to 13. Table III shows the result of linear fit from absorbance calibration plot of those three analyses, performed in the 1st prototype microfluidic mixer too. From those results, it can be obtained the similar conclusions as the ones obtained from the glucose analysis measurements.

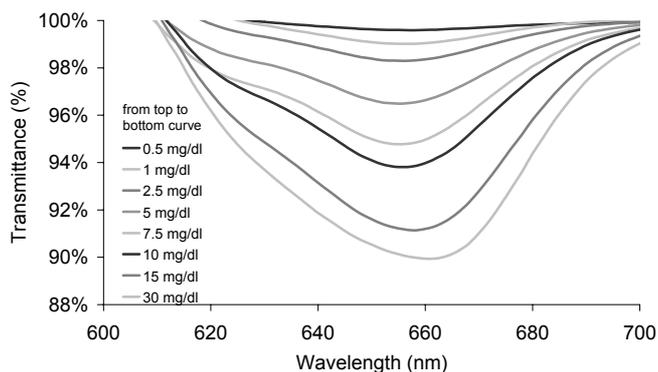


Fig. 8. Measured transmittance spectra for different calcium concentration in urine, with a photodiode placed underneath the microfluidic mixer (LP = 500 μ m).

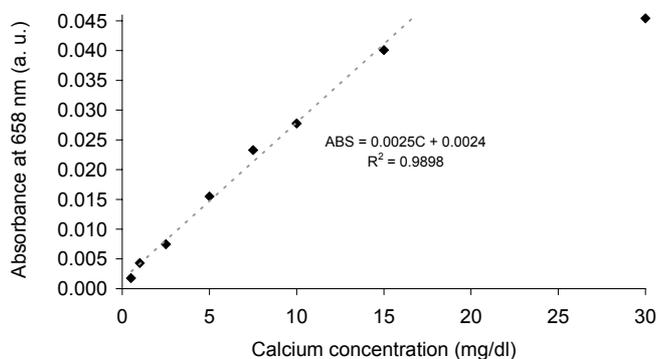


Fig. 9. Calibration curve for different calcium concentration in urine.

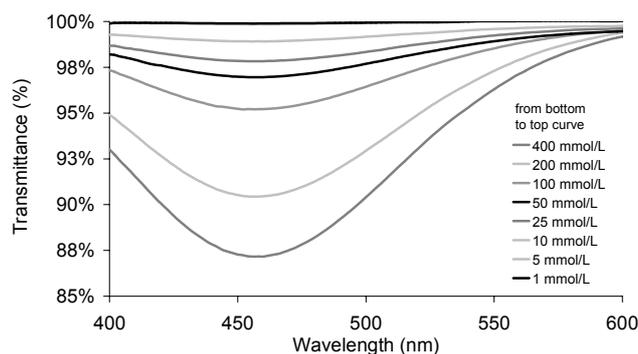


Fig. 10. Measured transmittance spectra for different chloride concentration in urine, with a photodiode placed underneath the mixer (LP = 500 μ m).

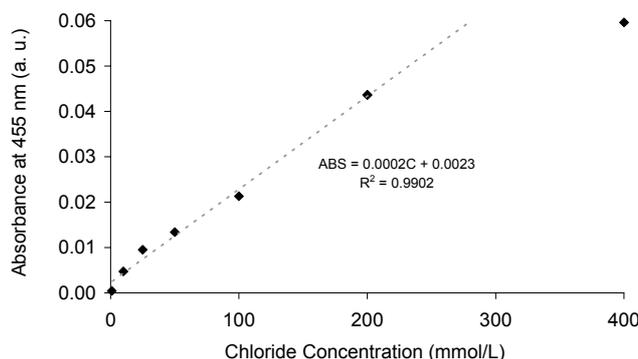


Fig. 11. Calibration curve for different chloride concentration in urine.

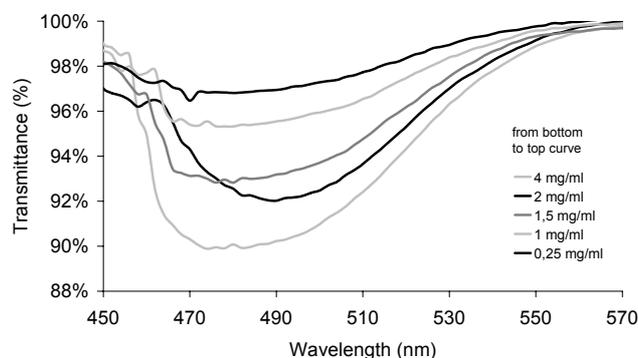


Fig. 12. Measured transmittance spectra for different creatinine concentration in urine, with a photodiode placed underneath the mixer (LP = 500 μ m).

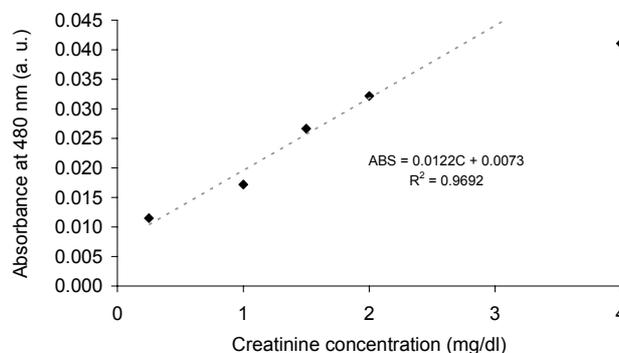


Fig. 13. Calibration curve for different creatinine concentration in urine.

TABLE III
RESULTING FIT PARAMETERS OF THE CALIBRATION CURVE FOR CALCIUM, CHLORIDE AND CREATININE CONCENTRATION IN URINE

Analytes (measured concentration range)	λ ABS max. (nm)	Linear concentration range	Slope (a.u.)dl/mg	Intercept (a.u.)	Correlation coefficient R ²	Δ Reproducibility of 10 assays
Calcium (0.5 – 30 mg/dl)	658	0.5 – 15 mg/dl	$(2.5 \pm 0.1) \times 10^{-3}$	$(2.4 \pm 1.0) \times 10^{-3}$	0.9898	15%
Chloride (1 – 400 mmol/L)	455	1 – 200 mmol/L	$(2.0 \pm 0.1) \times 10^{-4}$	$(2.3 \pm 1.5) \times 10^{-3}$	0.9902	10%
Creatinine (0.25 – 4 mg/dl)	480	0.25 – 2 mg/dl	$(1.2 \pm 0.2) \times 10^{-2}$	$(7.3 \pm 2.1) \times 10^{-3}$	0.9692	20%

VI. CONCLUSIONS

A SU-8 microfluidic mixer, integrated in a lab-on-a-chip device, for fluids handling and mixing was presented. It was developed for the simultaneous measurement of the concentration of four biomolecules in urine, by optical absorption. Preliminary simulations and experimental results have shown a homogeneous mixing obtained by diffusion before the detection zone. The sensitivity achieved in the measurements of glucose, calcium, chloride and creatinine 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 favorably with other conventional techniques. Moreover, this microfluidic mixer avoids the need of expensive readout optics and opens the door to low-cost disposable devices. Numerical simulations as well as experimental measurements for other biomolecules detection and also other biological fluids are on-going.

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