Advances in Science and Technology Vol. 57 (2008) pp 99-104
online at http://www.scientific.net

Online available since 2008/Sep/02

Ultrasonic Transducer based on β-PVDF for Fluidic Microagitation in a Lab-on-a-Chip Device

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Keywords: lab-on-a-chip, acoustic microagitation, β-PVDF, ITO, AZO.

Abstract. This paper describes a fully-integrated lab-on-a-chip device for testing and monitoring biochemical parameters in biological fluids. The major innovation of this microdevice is the application of an acoustic microagitation technique with automatic electronic control based on a β-PVDF piezoelectric polymer placed underneath the microfluidic structures. Experimental results regarding the influence of the thickness of the polymer on the reaction rate of biological fluids are presented. Moreover, the study of the transmittance curve of β-PVDF with transparent conductive electrodes is also presented. Transparent electrodes are a constraint once the polymer is incorporate underneath the reaction chamber due to the analytical measurement by spectrophotometry.

Introduction

Ingenious micromixing systems have been developed in order to overcome the limitations of mixing microflows in microfluidic devices reaching a complete and effective mixing in short times. Several approaches have been tried. MEMS (Micro Electro Mechanical Systems) based devices have been used, such as microvalves and micropumps [1]. However, these systems increase the cost of the system, the complexity of the control system and are difficult to integrate in a single-chip. Other approaches rely in long and complex channel topologies, that can be difficult to microfabricate and usually involve long transit mixing times, especially when the fluids diffusion coefficients are very small [2]. A different approach can be the use of acoustic waves. They have been used both to promote mixing [3] and to pump [4] fluids. One possible way to achieve these effects is using a piezoelectric material.

Poly(Vinylidene Fluoride) - PVDF - is still the polymeric material with the best electroactive properties. It combines the piro- and piezoelectric characteristics, with an excellent combination of processability, mechanical strength, lightness, moldability and low production cost. Additionally, PVDF is highly resistant to chemical agents and aging. While ceramic materials break easily and have hard and dense structures, PVDF is flexible, has a low density and can be easily produced into thin films (Table 1) [5].

Other notable features of the polymer are its low acoustic and mechanical impedance, crucial for generating the acoustic waves that produce the microagitation of the fluids, as it is the purpose of this study. The electromechanical coupling coefficient , an indication of the efficiency in the generation of acoustic waves though an electrical signal, is usually higher for ceramic materials. However, if the sound waves are propagated through fluids or plastics, most of the acoustic energy generated by the piezoceramic element is reflected in the boundary layer between the piezoelectric material and the propagation medium. Due to the low acoustic impedance of the fluids and plastic (from to $3 \times 10^6\ Kg/n$) and the high impedance of the ceramic, the coefficient of reflection

on the boundary layer is greater than 90%. Consequently, only a fraction of the acoustic energy generated by the piezoelectric element is transferred to the propagation medium. That's why piezoelectric polymer as the PVDF and its copolymers are a better option due to the low acoustic impedance [6].

Table 1: Different electromechanical properties of piezoelectric materials [5].

Parameters	PVDF	PZT	$BaTiO_3$	Units
Density	1.78	7.5	5.7	10^3 $kg/$
Permissivity ε_r	12	1200	1700	
Constant d_{31}	23	110	78	10^{-12} C
Constant g_{31}	216	10	5	10^{-3} Vm
Constant k_{31}	12	30	21	% a 1 kHz
Acoustic impedance	2.7	30	30	10^6 kg/m

Another interesting feature of the PVDF is its transparency. Indeed, in the case of this application, the analytical measurement method, by spectrophotometry, requires that the PVDF and the corresponding conductive electrodes, ITO (Indium Tin Oxide) or AZO (Aluminum doped Zinc Oxide), deposited in the reaction chamber are transparent to visible light. Thus, the knowledge of the transmission curves of these materials in the visible range of the electromagnetic spectrum is extremely important.

All those properties allow concluding that the incorporation of a piezoelectric film would be an advantage in lab-on-a-chip devices, leading to an improvement in the mixing process of fluids. Moreover, using the various properties of the PVDF material, it will work as a transducer that can generate sound waves under the stimulation of electrical signals. In this way, controlling the characteristic of the electric signals and the dimensions structure of the polymer, it is possible to modify its acoustic properties.

A lab-on-a-chip was developed for measuring the concentration of some biomolecules in urine samples by optical absorption [7]. It is composed by three parts in a multi-chip-module: a microfluidic system containing the microchannels fabricated using SU-8 technique [8]; an optical filtering system based on highly selective Fabry-Perot optical resonators [9] and a detection and readout system fabricated in a CMOS microelectronic process [10]. In this lab-on-a-chip, mixing of the samples with reagents was performed by diffusion, which leads to long transit times, especially when large biomolecules with small diffusion coefficients must be analyzed. Therefore, to be valuable for point of care testing and monitoring of a large set of molecules, the microfluidic die of the lab-on-a-chip requires a microagitation mechanism. As it is desirable that this mechanism does not require any external apparatus, internal moving parts or valves, acoustic microagitation by piezoelectric polymers is to be implemented.

Device Description

This paper describes the incorporation and validation of piezoelectric β-PVDF for use as a acoustic microagitatior in a fully-integrated disposable lab-on-a-chip for point of care testing and monitoring of biochemical parameters in biological fluids. With the deposition of the polymer underneath the microfluidics structures, acoustic microagitation can be achieved through electrical actuation, which leads to the enhancement of mixing and reaction time without moving parts. In this way, besides accelerated mixing time of the fluids, the device can be a point-of-care system with interesting characteristics such as portability, low cost and disposability. Furthermore, it has a completely automatic operation and uses optical absorption spectrophotometry as measurement analytical technique.

β-PVDF Polymer

The PVDF presents an unusual polymorphism in this class of materials, showing four different crystalline phases known as α, β, γ and δ [11]. The β-phase is the one which shows better properties to be applied in sensors, actuators and transducers, due to its higher piezo-, piro- and ferroelectric properties. This phase was obtained by stretching, at temperatures below 100ºC, the non-polar α-phase, obtained from the crystallization of PVDF from solution with N,N-Dimethyl Formamide and high temperature annealing [12]. The reason of stretching relation between the initial and the final length of the sample was from 4 to 7. After getting the electroactive β phase, the material must be activated by poling, that is, the application of an electric field poled in electric fields larger than 60 MV/m along the thickness direction. The conductive transparent electrodes of ITO and AZO were deposited by Physical Vapor Deposition.

Acoustic Agitation (*Quartz Wind*)

Piezoelectric materials convert the electrical energy into an acoustic wave, generating the movement of fluids under the effect of acoustic streaming, the *quartz wind* phenomena [13, 14]. The acoustic streaming can be categorized into two types: one can result from traveling waves on walls; in the case of *quartz wind*, the absorption of energy by the fluid itself results in an exponentially decaying acoustic intensity that generates a force per unit volume on a fluid equal to,

$$F = \frac{I}{c}\frac{e^{-x/l_\mu}}{l_\mu}. \quad (1)$$

where represent the acoustic intensity, is the sound velocity in the fluid and is the intensity absorption length in the fluid or the inverse of the absorption coefficient. The force is in the direction of propagation on the acoustic radiation [15].

Measurement Analytical Technique

In clinical diagnostics, the colorimetric detection by optical absorption is the spectrophotometric analysis technique most commonly used to determine the concentration of a particular biochemical parameter in biological fluid samples. As many of the analytes of interest do not have chromophores that absorb light in the visible range, specific chemical reagents are available to transform these analytes into colored products with adequate absorbance [16]. The mixture shows a maximum absorbance at a specific wavelength, depending on the characteristic of the biomolecule and reagent (Fig. 1).

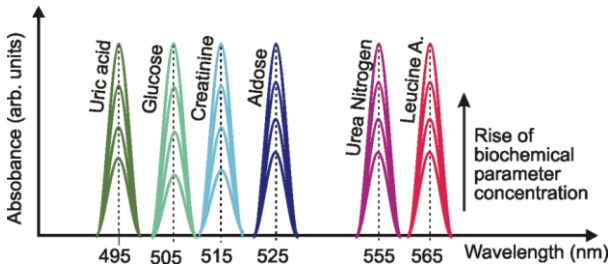

Fig. 1: Absorption spectra for some biochemical parameters with different concentrations in urine.

Biosystem Operation

The lab-on-a-chip is composed by two dies: the microfluidic die and the detection die (Fig. 2). The microfluidic die includes the microchannels and the reaction chambers. Three cuvettes are needed for each analysis: one for the chemical reagent, in order to obtain the baseline reference; other for the mixture of the sample plus the reagent, to perform the analysis of the colored mixed solution; and a third one with a standard sample with a well-known concentration of the

biochemical parameter that is being analyzed, for the calibration of the biochemical parameter concentration. The lab-on-a-chip is fabricated using a photoplastic material, SU-8. The channels and the reaction chambers are deposited and/or coated by the corresponding electrodes and the piezoelectric β-PVDF (Fig. 3a). This structure will produce the needed acoustic vibration along the channels. By applying electrical alternating voltages to the electrical contacts of the β-PVDF that are placed along the microchannels and inside the reaction chambers, mechanical oscillations improve the motion, mixing and the reaction of the fluids.

The detection die includes the detectors and the electronics for signal actuation and detection, all fabricated in CMOS technology (Fig. 3b). Specifically, it comprises the photodetectors, its readout electronics and the control electronics for the microagitation. On this die and above the photodetectors, there are several high-selective band-pass optical filters, deposited by Ion Beam Deposition, that select the wavelength according to the several biomolecules into analysis. This optical filtering system allows the use of a non-calibrated external polychromatic light source, being the reliability of the measurements assured by the fact that the lab-on-a-chip compensates the fluctuations associated to the use of that light source, once it detects simultaneously three optical signals: the fluidic samples, the baseline reference and the calibration control.

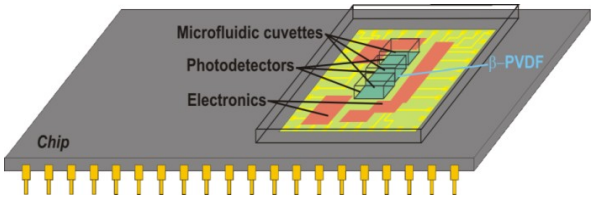

Fig. 2: Schematic representation of the lab-on-a-chip structure.

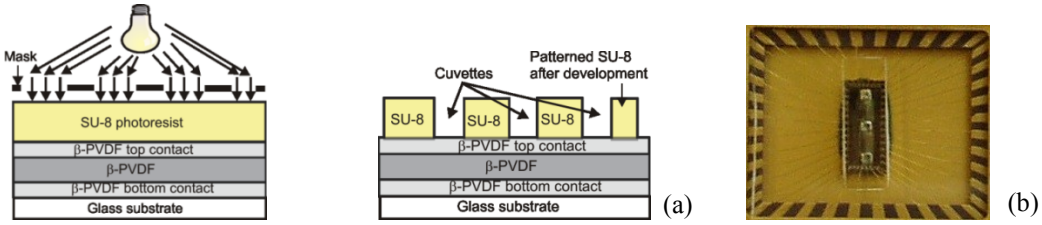

Fig. 3: (a) Fabrication sequence of the microfluidic cuvettes of the prototype: deposition, spin coating, soft bake, UV exposure and development of the SU-8; (b) Picture of the detection die with the reaction chambers.

Experimental Results

The evaluation of the mixing process was carried out quantitatively and qualitatively. To evaluate qualitatively the mixing performance, an experiment was prepared in order to visualize the mixture. Two cuvettes were set for the chemical reaction between a solution of Sodium Hydroxide, Sucrose and Potassium Permanganate. In one of the cuvettes, microagitation was performed using sinusoidal signals of 10 V amplitude, with 15 MHz frequency on the β-PVDF transducer. The Figure 4 shows the mixing differences between both cuvettes. With the application of acoustic microagitation the mixing reaction occurs faster, being the time necessary to obtain the complete reaction approximately half (102 s) of the complete mixing time without oscillation (209 s).

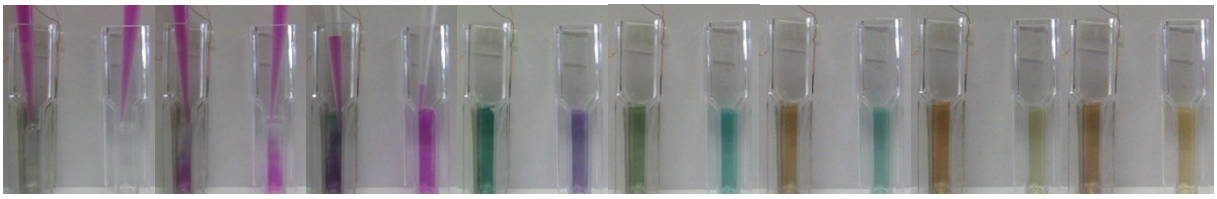

Fig. 4: Evolution of the mixture with and without acoustic microagitation, left and right cuvette respectively.

The quantitative analysis was performed measuring the uric acid concentration in urine using the Sigma Diagnostic Kit and standards of urine with 15 mg/dl of uric acid concentration. The reagent reacts with a sample of urine acid in 50:1 ratio, and produces a pink color whose intensity is proportional to the uric acid concentration with a maximum absorption at 495 nm [17].

The manual procedure requires a delicate inversion of the cuvette containing the mixture. After agitation for approximately 5 minutes at room temperature, the mixing is complete and homogeneous. Without any agitation and due to the high diffusion coefficient of uric acid concentration, the complete mixture takes approximately 15 minutes at room temperature. In clinical laboratories, the macroscopic equipments have mechanical agitation of the cuvettes for improving mixing and reducing the reaction time.

Previous studies have shown that the mixing occurs more quickly with a frequency of 1 kHz compared to 300 Hz and 10 kHz (one third of the time needed without microagitation for 1 kHz, two fifth for 10Khz and four ninth for 300 Hz) [18]. In this way, the microagitation was set using sinusoidal signals of 3.3 V amplitude and 1 kHz frequency. The evaluation of the mixing process was carried out for two thickness of β-PVDF (28 μm and 110 μm) both with the same area (2.4 cm^2). The system was calibrated for an absorbance of 0 a.u. filling the cuvette with deionized water. The results obtained with the spectrophotometer (Unicam Helios Gamma&Deta) are shown in Fig. 5a. As can be seen, the mixing occurs in a faster way with a thin thickness, being the time necessary to obtain the complete mixing (0,535 a.u.) for 28 μm tickness only one sixth (245 s) of the complete mixing time for 110 μm thickness (304 s) and half of the complete mixing time without oscillation (513 s).

Moreover, it was measured the transmittance curve of β-PVDF with ITO and AZO electrodes for evaluating which one as the better transmittance, once the polymer is deposited underneath the reaction chamber where the analytical measurements occur. For that, a film of 28 μm thick β-PVDF was used with electrodes of ITO (110 nm thick) and another with AZO (115 nm thick) deposited on both sides of the polymer. The results are shown in Fig. 5b. As it can be seen, better results are obtained in the case of β-PVDF with electrodes of AZO, the transmittance curve has higher values and is approximately constant over the visible spectrum. Indeed, for the sample with ITO, the transmittance decreases dramatically when approaching the UV wavelengths, which is disadvantageous in the case of biomolecules analysis with a maximum absorbance in this area. However, the transmittance curve of both electrodes can be improved by optimizing the processing and deposition conditions.

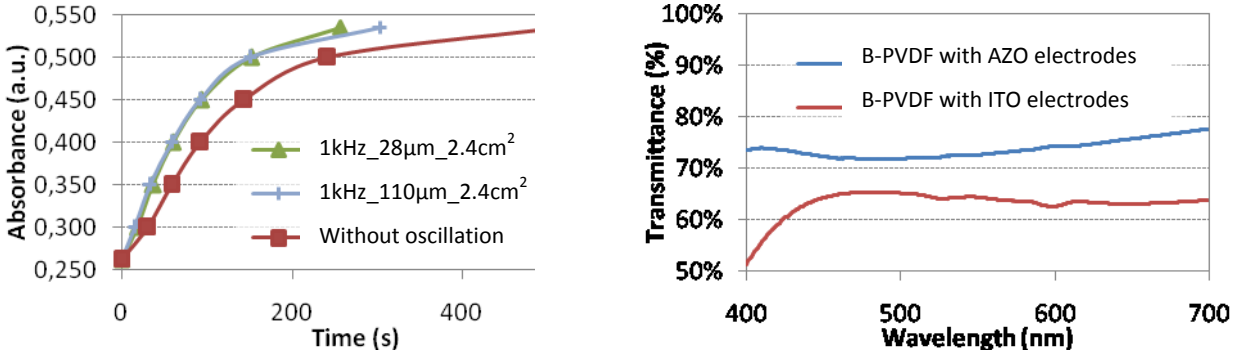

Fig. 5: (a) Evolution of the absorbance at 495 nm for 15 mg/dl of uric acid concentration as a function of time, for the frequency of 1 kHz and two thickness; (b) Transmittance curve of the β-PVDF with ITO and AZO electrodes in the visible area of the electromagnetic spectrum.

Conclusions

The application of acoustic microagitation through the β-PVDF piezoelectric polymer is advantageous when two or more fluids need to be mixed. It accelerates the mixing time resulting in a quicker complete and homogeneous reaction of the reactants and improving the global performance of the analysis that is being performed. With a 28 μm thickness of β-PVDF, the mixing

time is less when compared with a 110 μm thick. Moreover, in the case of the deposition of the polymer underneath the reaction chamber of the lab-on-a-chip, the β-PVDF with AZO electrodes is more adequate comparatively to the β-PVDF with ITO electrodes, since it offers a higher and almost constant transmittance curve in the visible area of the electromagnestic spectrum.

As a conclusion, it can be stated that, for descreasing the size and complexity of the device, acoustic microagitation becomes a preferred technology for effective mixing.

Acknowledgment

Support for this research was provided by the Portuguese Foundation for Science and Technology (grants PTDC/BIO/70017/2006, PTDC/CTM/69362/2006 and POCI/CTM/59425/2004).

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