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# LTCC Microfluidic Systems for Biochemical Diagnosis

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This paper presents design, fabrication and testing of three LTCC (Low Temperature Co-fired Ceramics) based microfluidic systems. These microdevices are: enzymatic microreactor for urea determination, potentiometric sensor with ion selective electrodes (ISE) based array sensitive to potassium ions and amperometric glucose sensor. Performance of the presented LTCC-based microfluidic systems has been tested. All ceramic microdevices have revealed high output signal and large detection range. The properties of the presented LTCC-based microfluidic systems are comparable with similar ones made of silicon. Obtained results has shown that presented ceramic microsystems can work as a stand-alone device or can be integrated into a more sophisticated micro analysis system for *in vivo* or *in vitro* monitoring of various (bio)chemical compounds.

K e y w o r d s: LTCC (Low Temperature Co-fired Ceramics), thick-film, numerical modelling, microreactor, sensor

## 1. Introduction

Modern analytical procedures which are applied in chemistry, biology or medicine consist of several steps: sample collection, carrying out appropriate (bio)chemical reaction, product separation and detection of the analyte. Classical instrumental analysis is characterized by relatively long time of detection, considerable reagents consumption and large amount of wastes. All these disadvantages seem to be eliminated by use of micro-total analysis systems (μTAS) or lab-on-chip (LOC) devices [1]. These miniature devices work with liquid samples in the micro- or even nanoliter

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scale [2]. Thanks to this, they are characterized by small size, short response time, high sensitivity and good selectivity. Moreover, they produce less wastes in comparison with classical laboratory equipment used in analytical chemistry. Therefore, microfluidic systems finds practical applications in analytical diagnosis and continuous monitoring of various biochemical parameters. Quite recently, LOC and μTAS devices were manufactured mainly using silicon micromachining technology. However, in accordance with the newest trends, cheaper technologies and materials are applied. Modern microfluidic systems are quite often manufactured using relatively cheap polymers [3, 4], PCB (Printed Circuit Board) [5, 6] and LTCC (Low Temperature Co-fired Ceramics) [7, 8] technologies. In comparison with the PCB technology, the advantages of the LTCC technique, are the following: chemical inactivity, chemical resistance, good thermal conductivity, high temperature stability. Moreover, the LTCC tapes can easily be cut into desired form in the way to accomplish both mechanical and electrical functions under a single ceramic module. The main profit of the LTCC technique is possibility of integration of fluidic structures, passive components, sensors, actuators, electronics and package into a multilayer module. The LTC ceramic can be easily bonded to other materials e.g. silicon [9], glass [10] or polymers [11]. Thanks to these advantages, the LTCC has found practical application as flow sensors, electrochemical biosensors, microanalyzers, micromixers, microreactors and polymerase chain reaction (PCR) devices [12–15].

This paper presents design, fabrication and measurements of three ceramic-based microfluidic systems for in vivo monitoring of various chemical compounds. The enzymatic microreactor for urea determination, potentiometric sensor with ion selective electrode (ISE) based array sensitive to potassium and ammonium ions and amperometric glucose sensor were fabricated using the LTCC microelectronic technology, which has a few advantages over other microfabrication processes. The ceramic microfluidic systems performance was evaluated experimentally. All presented LTCC-based devices revealed high output signal and large detection range comparable with similar ones made of silicon.

## 2. LTCC Technology

The LTCC (Low Temperature Co-fired Ceramics) microelectronic technology is commonly used for hybrid circuits fabrication. In the past it was used to produce multilayer devices for telecommunication, automotive and aerospace application. Recently, this technique is also applied for production of sensors, actuators and microsystems. The LTCC was developed in the end of 1980s. A traditional structure consists of several dielectric tapes (alumina filled glasses), connecting vias, surface and buried conducting lines and passive components (resistors, capacitors, inductors). A flow-chart of the LTCC multilayer module fabrication process is presented in Fig. 1.

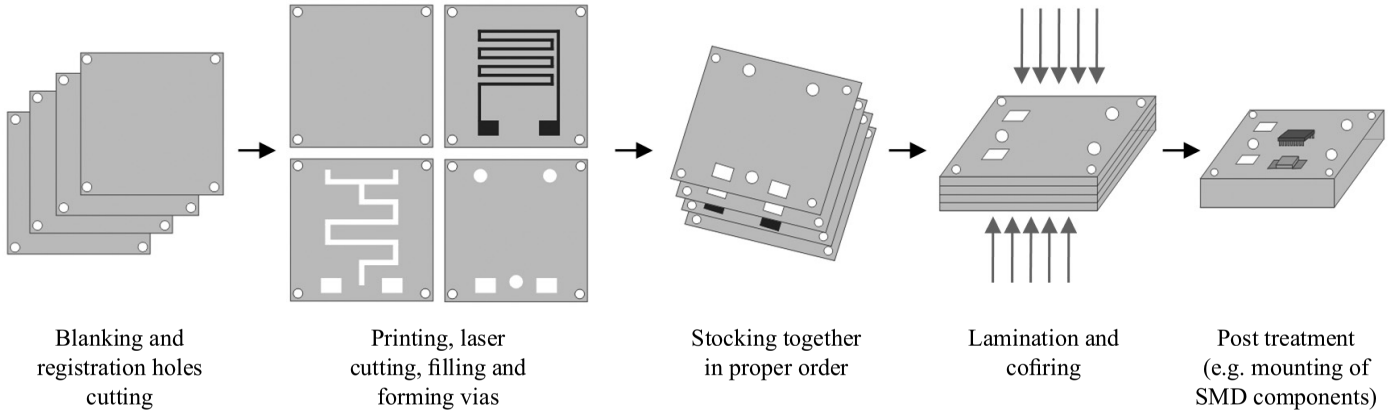


**Fig. 1.** Flow-chart of the LTCC process

There are few substantial steps in the process. First the "green" ceramic tapes are blanked to specific size and then registration and orientation holes are cut out. Next vias are made in each individual LTCC tape. The vias, registration and orientation holes are cut out using CNC (Computer Numerical Control) punching machine, laser or photo patterning method. Then the via holes are filled with conductor (Ag or Au) paste. In the next step, conductive lines and passive components (resistors, capacitors and inductors) are deposited on the ceramic tape using standard screen-printing or ink-jet printing method. After printing fluidic channels, cavities are cut out using mechanical puncher or laser. The individual LTCC tapes are stacked together in a proper order and laminated in an uniaxial or isostatic press. During lamination process the LTCC tapes are joined together and they form a multilayer module. Typical lamination process takes place under high pressure (up to 30 MPa) and elevated temperature (up to 90°C) for 1–20 minutes. Finally the LTCC module is co-fired in a special two-step thermal profile with a maximum temperature of 850°C in air atmosphere. Afterwards active and passive components can be placed on the LTCC module's surface using standard wire bonding, SMT (Surface Mounting Technology) or flip-chip techniques.

## 3. LTCC Microfluidic Systems

The LTCC and related technologies are almost ideal for fabrication of biosensors, microreactors or other sub-systems for integrated μTAS or LOC devices due to its inherent features: chemical inactivity, very good chemical resistance, biocompatibility, hermeticity and easy 3D structuration. In this paper, three exemplary LTCC-based microfluidic systems for biochemical diagnosis are presented.

### 3.1. Enzymatic Microreactor for Urea Determination

A batch type flow-through enzymatic microreactor with an integrated heater was made in the LTCC technology. The presented construction of the microreactor was based upon similar one made of silicon [16]. Schematic view of the LTCC enzymatic microreactor is shown in Fig. 2. The ceramic-based microdevice consists of two cavities separated by a threshold. The batch in a form of porous glass beads with immobilized enzyme (urease) was placed in a larger compartment of the microreactor. The principle of the microfluidic system operation is based on hydrolysis of urea catalyzed by urease:

$$CO(NH_2)_2 + 3H_2O \xrightarrow{urease} CO_2 + 2NH_4^+ + 2OH^- \quad (1)$$

One of the reaction (1) products is a hydroxyl group which can be used for indirect determination of urea (pH measurements) in the investigated sample. The real structure of the enzymatic microreactor was made with use of the LTCC technology and nine

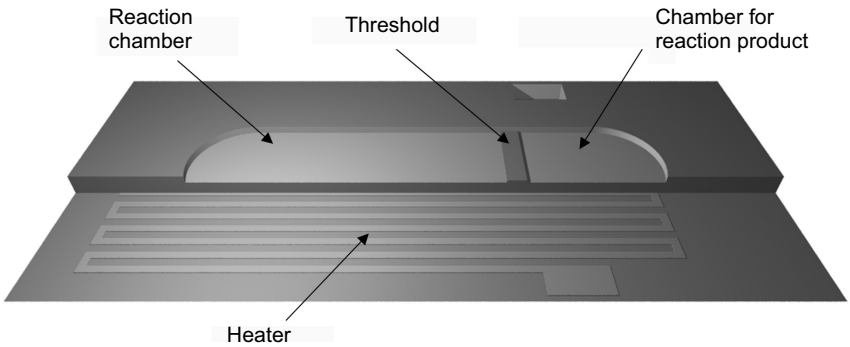


**Fig. 2.** Schematic view of the enzymatic microreactor

layers of DP951 P2 tape. After firing the thickness of each layer was equal 137 μm. The cuts for chambers and electrode terminals were made from "green" LTCC tapes using a Nd-YAG laser. After laser patterning a platinum heater and a thick-film temperature sensor were screen-printed through 325 mesh steel screen. At the end all LTCC layers were laminated and co-fired in a box furnace (Nabertherm L3/S) with a standard two-step thermal profile with maximum temperature equal to 850°C.

The measurements of the device was performed in a closed loop flow-through system, where the sample was circulated through the microreactor. The pH changes resulted of hydrolysis of urea catalyzed by urease (1) were measured by the micro pH sensor placed in the sample. In 10-minute time intervals concentration of urea in the sample was changed by a standard addition method. The measurements were performed in 5 mM phosphate buffer containing 0.1 M sodium chloride of initial pH 6.1. Calibration curve for enzymatic microreactor is shown in Fig. 3. For the microreactor loaded with enzyme immobilized onto pours glass beads, the output

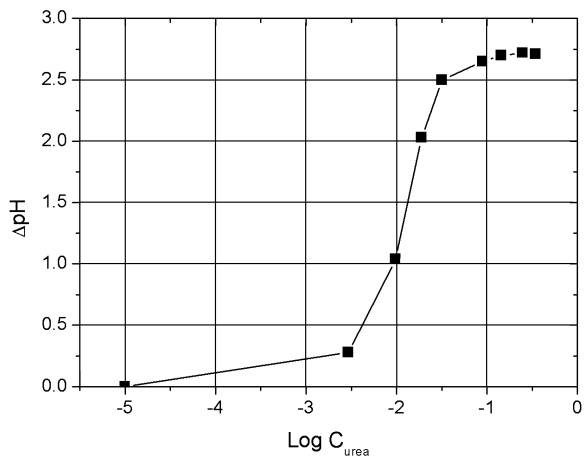


**Fig. 3.** Calibration curve for enzymatic microreactor with urease immobilized onto glass beads ($C_{urea}$ – concentration of the urea)

signal was very high – ca. 2.5 pH units – and comparable with output signal obtained for a silicon-based microreactor reported in [16].

### 3.2. Potentometric Sensor with Ion Selective Electrode (ISE) Based Array

The LTCC-based potentiometric sensor with an integrated ISE array consists of fluidic channel with rounded corners and four silver electrodes. The ceramic sensor was dedicated to potassium ions determination. The principle of its operation was based on ISE electrode. Potential of the ion selective electrode was measured against a classic type reference electrode (RE). Difference between potentials of the ISE and RE is proportional to concentration of a specific ion in a test solution. The potential of the ISE electrode, according to Nikolsky equation is defined as:

$$E_{ISE} = E^0 + \frac{RT}{nF} \ln\left( c_i + \sum_{j=1}^{n} K_{ij} c_j^{\,n/z} \right) \tag{2}$$

where $E_{ISE}$ is a potential of the ISE electrode (V), $E^0$ is a standard electrode potential (V), $R$ is the universal gas constant (8.314J $K^{-1}$ $mol^{-1}$), $T$ is the absolute temperature (K), $F$ is the Faraday constant (9.648 x $10^4$ C $mol^{-1}$), $K_{ij}$ is the selectivity coefficient for a primary ion $j$ over an interfering ion $i$, $z$ is the valence of the primary ion $j$, $n$ is the valence of the interfering ion $j$.

Schematic view of the LTCC-based potentiometric sensor with a ion selective electrode (ISE) based array is presented in Fig. 4. It consists of a fluidic channel and four chambers for ISE electrodes. Each of chamber consists of three cavities for ion-selective membrane, hydrogel and silver chloride layer. The real structure of the sensor was manufactured of nine DP 951 P2 tapes. The fluidic channels and the cavities were cut out in the ceramic layers using a Nd-YAG laser before firing. The ISE-array located inside of the LTCC module was deposited with use of silver (DP 6145) ink by screen printing method. After laser cutting and screen printing, all ceramic tapes were laminated and co-fired ($T_{max}$ = 850°C).

The silver/silver chloride electrodes were made by electrochemical chloriding of screen printed silver pads in 0.1 M potassium chloride (+1.5 V vs. Pt electrode, until total decline of the current for at least 15 minutes). After chloriding, the electrodes were chemically modified to form a intermediate hygrogel layer and a ion selective membrane. The hydrogel layer of modified poly(2-hydroxy-ethyl)methacrylate (polyHEMA) and the membrane were deposited with a micropipette. A quality of the microfluidic sensor was examined experimentally. Measurements were taken in 0.1 M sodium chloride. In the testing solution concentration of the potassium ions was changed gradually from: 0.02 mM to 92.5 mM, every 200 seconds. The measurement data were recorded in 2 seconds time intervals. The time responses and the corresponding calibration curve is shown in Fig. 5. The presented potentiometric sensor was destined for integration with the LTCC enzymatic microreactor described in previous section.

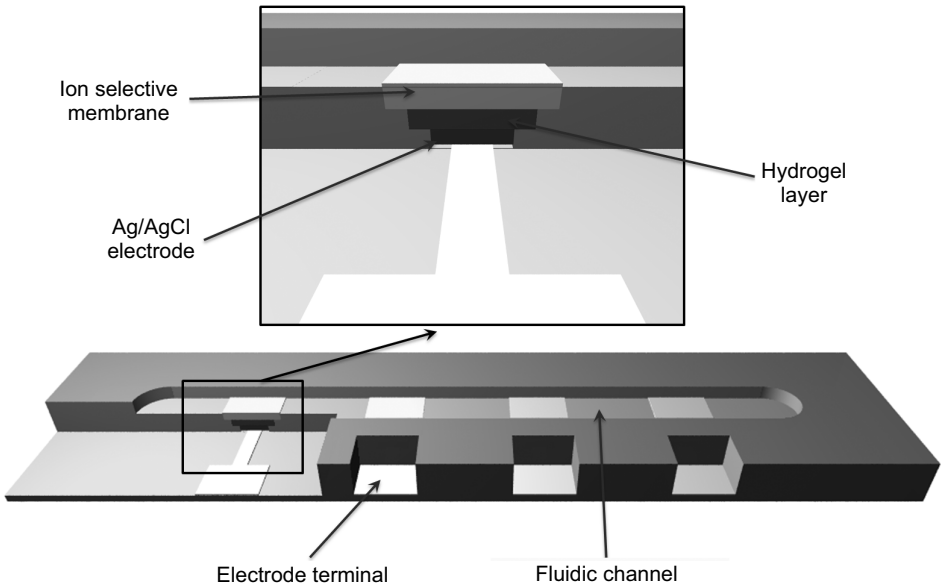


**Fig. 4.** Schematic view of the LTCC-based potentiometric sensor

### 3.3. Amperometric Sensor for Continuous Glucose Monitoring

A flow-through sensor with an integrated microdialysis tube and three thick-film electrodes for amperometric detection was made in the LTCC technology. The schematic view of the ceramic-based amperometric sensor is shown in Fig. 6.

The presented microfluidic device was destined for continuous glucose monitoring. Construction of the flow-through sensor is based on semi-permeable dialysis tubing built-in a microreaction cell filled with an enzyme solution. The principle of its operations is based on size selective diffusion of glucose. Glucose diffuses from the test sample through walls of the semi-permeable dialysis tube to the microreaction cell. The microreaction cell is filled with a buffer solution containing free enzyme – glucose oxidase (GOx). In the presence of the GOx glucose is oxidized and gluconoactone and hydrogen peroxide are produced:

$$glucose + O_2 + H_2O \xrightarrow{GOx} gluconolactone + H_2O_2 \tag{3}$$

One of the reaction products is hydrogen peroxide which is detected amperometically during its oxidation at a surface of the working electrode:

$$H_2O_2 \longrightarrow O_2 + 2H^+ + 2e^- \tag{4}$$

The current generated in the reaction (4) is proportional to the glucose concentration.

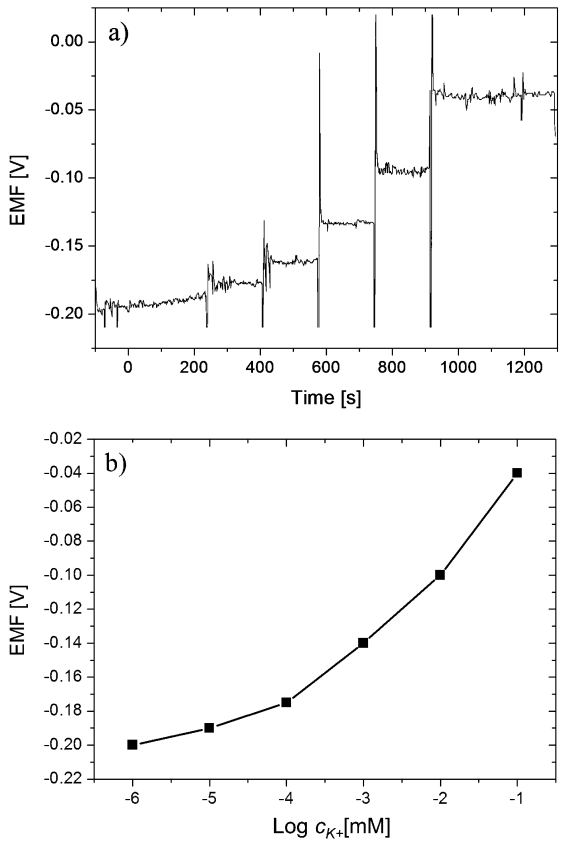


**Fig. 5.** Time responses (a) and the corresponding calibration curve (b) of the LTCC potentiometric sensor ($C_{K+}$ - concentration of the potassium ions)

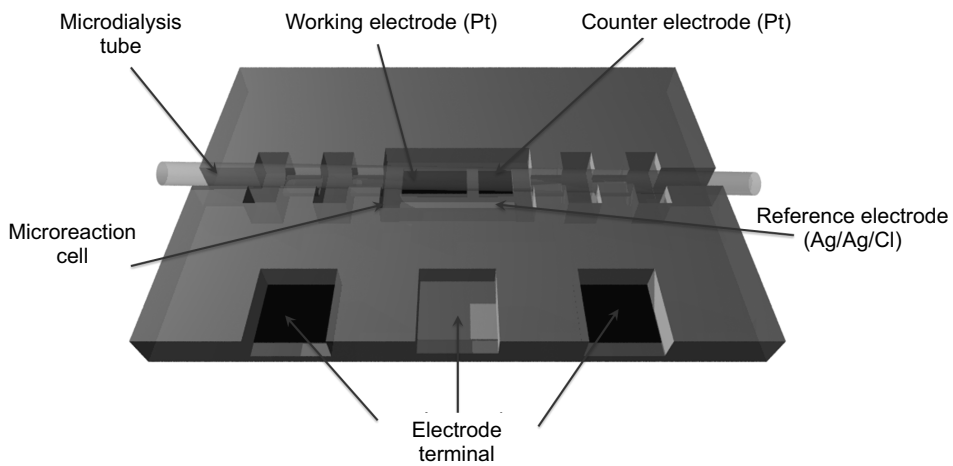


**Fig. 6.** Schematic view of the amperometric glucose sensor

The presented amperometric glucose sensor was made of ten DP 951 P2 tapes. Cuts for the microreaction cell, channel for the microdialysis tube and the electrode terminals were made with a Nd-YAG laser. Platinum ink was used for construction of the working and counter electrodes while silver paste was used for the reference electrode.

The ceramic biosensor was tested in a flow-through system in a continuous mode. The microreaction cell was filled with glucose oxidase solution (15.5 U/mL of GOx in the phosphate buffer solution). The glucose concentration was changed in range from 0.5 mM to 12.5 mM. In regular 300-seconds time intervals the concentration of glucose in the sample solution was gradually changed. The LTCC amperometric sensor was operated as a three-electrode system. The potential of the working electrode was held constant at +650 mV versus an Ag/AgCl reference electrode. The oxidation current at the working electrode was measured with use of a potentiostat PAR VMP2/Z. All measurements were performed at room temperature. The resulting dynamic response and the corresponding calibration curve are presented in Fig. 7.

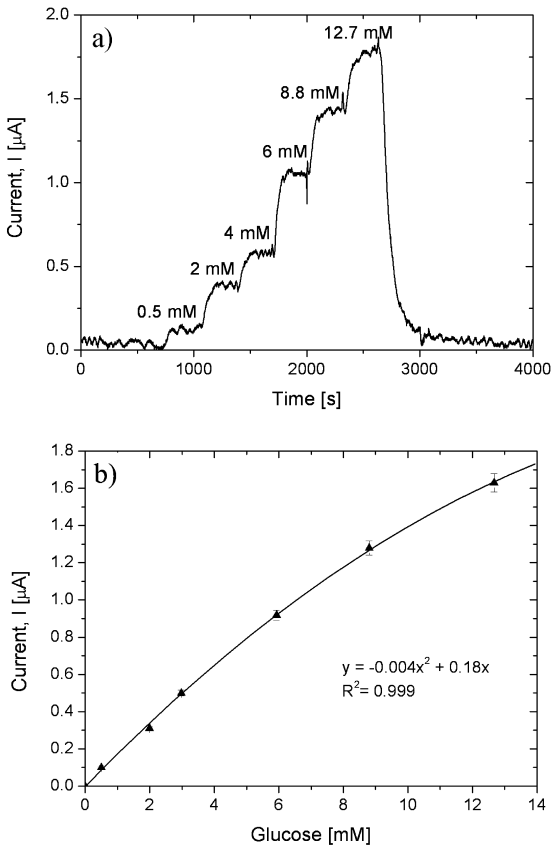


**Fig. 7.** (a) dynamic response and (b) calibration curve of the LTCC glucose sensor

A linear response of the LTCC-based amperometric sensor to glucose was up to 12.5 mM. The presented ceramic sensor has about ten times higher sensitivity (ca. 135 nA/mM) in comparison with a similar construction of sensor made of silicon (ca. 16 nA/mM) [17].

## 4. Conclusions

Realization of various microfluidic systems for biochemical diagnosis using LTCC microelectronic technology has been presented and discussed. The LTCC-based biosensors and enzymatic microreactor reveal high output signal and large detection range. Their properties are comparable with similar microsystems made in other popular technologies. The low temperature co-fired ceramic presents a very promising alternative for fabrication of the microfluidic systems. Its inherent features (easy three dimensional structuration, matching of thermal expansion coefficient with silicon, possibility of fluidic structures and thick-film electrical lines, electrodes, heaters integration in one module) and very good properties (chemical inactivity, chemical resistance, biocompatibility [18]) of the presented ceramic-based microfluidic systems have shown that the LTCC technology has a potential for implementation in μTAS and LOC devices.

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