

Microfabrication of a Free Flow Electrophoresis on Low Temperature Co-Fired Ceramics Technology

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Abstract.

In this paper, a study of the power and impulse frequency influences on the microfabrication fluidic structures by infra-red laser ablation in low-temperature co-fired ceramics (LTCC) substrates is presented. LTCC technology, which has been commonly used for electronic applications, is presented as a useful alternative to construct continuous flow analytical microsystems. A μ -FFE device, with electric field generation by electrostatic Induction in LTCC technology fabrication is described. In order to use optical detection in microfluidic LTCC systems, a novel gasket like LTCC-PDMS-PMMA sealing system is presented.

Keywords: LTCC; Microfluidics; Microfabrication; FFE; μ -TAS

Introduction

Over the past decade, advances in the miniaturization field, especially those related to microfluidics, have been intensively applied to the development of analytical instrumentation in order to solve chemical and biological problems. The (bio)analytical and chemistry field has become one of the most important users of the scaling technologies due to their inherent advantages, such as low cost (mass production), reduction of sample and reagents consumption, higher analysis frequency, compact design, ease of operation, and portability.

Since the 1990s, the combination of the miniaturization-integration concepts has helped to establish the conceptual bases to develop a new generation of analytical instrumentation, the so-called micro total analysis systems (μ -TAS). These μ -TAS are made up of miniaturized

devices able to integrate the whole analytical process [1]. The fusion of the microelectronic technologies with the continuous flow methodological concepts, widely applied in analytical chemistry, provides the tools needed to obtain this kind of analytical instrumentation. Unfortunately, the high variety of key components needed to design μ -TAS has delayed the attainment of the considered goals. Among the functional elements needed to give the solution to the widest range of analytical problems, are microfluidic structures as well as their connections, pumps and valves, injection elements, reactors, filters, separation and preconcentration devices and microsensors [2]

Glass and silicon have been the most widely used materials with miniaturization purposes; this is in part due to their versatility and chemical inertness, the relatively straightforward fabrication, and the easy

integration of optical detection. On the other hand, plastic devices possess a number of advantages over glass and silicon technology, which include the speed of manufacture and lower fabrication cost.

The ideal fabrication technology would take advantage of the multilayer construction concept used by polymer-based techniques, and it would overcome the sealing problems, following the silicon technologies approach. Some other characteristics, such as realistic cost without the need of special fabrication conditions (clean rooms) would be of great interest in order to achieve mass production.

Low-temperature co-fired ceramics (LTCC) [3] technology meets all these requirements showing unique capabilities to conjugate the most important features demanded by analytical microsystems designers: easy construction of complex three-dimensional structures by means of a multilayer method, monolithic integration of system components and rapid prototyping at low cost

Since the introduction of free-flow electrophoresis (FFE) in the 1960s [4], this separation method has found a permanent position among analytical and preparative methods in biochemistry and chemistry for the separation of, cells, organelles, peptides, proteins, inorganic, and organic compounds [5]. In FFE a narrow sample stream is continuously introduced into a separation chamber filled with running buffer. An electric field is applied perpendicular to the sample stream. Consequently, analyte molecules are affected by two perpendicular vectors causing a movement at an angle to the flow

and thus resulting in a geometrically speaking two dimensional separation.

The miniaturization of FFE implies several advantages especially considering sample volume and separation speed. In contrast to the tens of milliliters of sample consumed by conventional large-scale FFE devices, μ -FFE systems require only tens of nanoliters up to hundreds of microliters of sample. Furthermore, instead of residence times of up to tens of minutes, μ -FFE devices separate within several seconds[4]. The greatest challenge in the fabrication of μ -FFE devices is the method of voltage transfer to form the electric field in the separation chamber. In conventional bench-top FFE instruments electrodes are separated from the separation department by a membrane serving as an electrolyte bridge. In microfabrication, the incorporation of such a membrane is difficult. Several approaches have been presented for microfluidic devices. Raymond [6] developed a μ -FFE chip that operated with closed side channels. This FFE microchip had a dense array of microchannels, acting as a membrane, separating the electrodes from the actual separation chamber. This liquid-filled channel array formed a high hydrodynamic resistance for the pressure-driven fluid, allowing electrical contact. In this design, 60% of the applied voltage reached the separation chamber generating the electric field. However, optimization between side channel and separation chamber flow was not achieved, negatively influencing the separation. Zhang [7] described a μ -FFE device where 108 fine channels connected the separation chamber and the remote open electrode

reservoir on each side serving as an electrolyte bridge. Like a membrane, these fine channels allowed electric conduction while at the same time reduced pressure flow between the electrode reservoirs and the separation chamber. The disadvantage of this layout was the small total cross-section of the connection channels causing a high ohmic resistance and thus a high voltage drop in the channels. Only 4.45% of the applied voltage reached the separation chamber generating the electric field across the compartment. Fonslow [8] presented a FFE device with a shallow separation region (20 mm deep) and roughly four times deeper closed electrode beds, completely avoiding side channel arrays or membrane equivalents. The flow rate through the electrode channels could now be significantly higher, effectively removing electrolysis products without disrupting the flow pattern in the shallow separation channel. In this approach 91 % of the applied voltage reached the separation chamber. Kohlheyer [9] integrated photo-polymerised, ion permeable membranes to hydrodynamically isolate the separation compartment from the side electrodes. With such a device they achieved 63% use of the applied voltage. However, the device is more laborious to fabricate than the above mentioned approaches and ion permeable membranes are mechanically fragile. Janasek [10] described a μ -FFE device with a different approach. In this device the electric field is generated by electrostatic induction across an insulating wall. Electrostatic induction is based on the displacement of charges across an insulator by dipole orientation. When a potential is

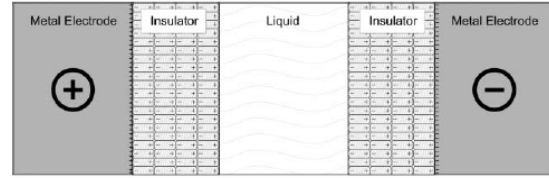


Fig. 1: Principle of electrostatic induction by charge displacement caused by dipole orientation

applied between the electrodes, like a chain, the dipoles transfer the charges from the surface of the electrode to the distant surface of the insulator. Thus, the insulator–liquid interface has the same polarity as the metal electrode. The displaced charges at the anodic and cathodic side of the separation compartment generate the electric field which causes electrophoresis. Although the polarity of the other side of the insulator is the same as the metal electrode, no current flows resulting in no electrolysis, meaning no bubbles formation, one of the major problems in μ -FFE. The device could be described as a capacitor. (Figure 1). The amount of charges Q depends on the specific characteristic of the insulator, ϵ_r :

$$Q=UC \quad (1)$$

$$C=(\epsilon_0\epsilon_rA)/d \quad (2)$$

where U is the applied voltage, C is the capacity of the barrier ϵ_0 is the absolute permittivity ϵ_r is the relative permittivity of the barrier A is the area of the barrier and d the distance of the barrier.

The device efficiency depends on the separation chamber geometry, the isolator layer thickness and the dielectric properties of the dielectric layer.

In Janasek’s design, 50 % of the applied voltage reached the separation chamber, using a 146 μm wide glass isolating wall with dielectric constant between 5 and 16.

This approach is very interesting for LTCC based devices. Being LTCC a dielectric material with a dielectric constant of 7,8 constant, there are a number of dielectric compatible ceramic pastes, that can be used to construct such an isolating layer, with dielectric constant that can reach 3000. In the future this could originate an enormous breakthrough in terms of separation resolution and low voltage usage. However in order to fabricate a μ -FFE device in LTCC, based on Janasek's design, is necessary to have micrometric fabrication control. LTCC substrates are usually cut by means of milling machines. Although suited for electronic devices fabrication, this contact technique, originates problems in the fabrication of microfluidic elements, due to the fragility of LTCC unfired tapes, and limits microfluids designs to the existing cutting tools to the order hundreds of micrometers. The use of a non-contact fabrication technique, such as laser ablation would allow the fabrication of complex microfluidics elements. We present a study on the influence of power and impulse frequency in the fabrication of microchannels in LTCC through infra-red laser ablation.

Other problem concerning the use of LTCC as a microfluidic substrate is its non transparency. Although do to ceramic layers characteristics, a compact and easy integration between fluidic, mechanical and electrical components can be achieved, without having structural problems related to the lack of sealing between layers or components, the use of optical detection systems, require post fabrication sealing of

the LTCC microchannels with transparent materials. Some of the methods used to join dissimilar materials include epoxies or simple gaskets. Epoxies promise a permanent bond between components, but the occurrence of flow channel occlusion during epoxy application, component assembly, and resin curing is likely as the excess of epoxy is squeezed from between the two surfaces to be joined. Using the elastomeric properties of polydimethylsiloxane (PDMS), we present a novel, low-cost, modular, LTCC-PDMS-PMMA gasket like sealing technique, that allow the use of optical detection systems in LTCC based microfluidic systems.

2 Experimental sections

2.1 Reagents

All reagents used, of analytical grade were obtained from Fluka. 1M solution of phenol red and 1M solution of methyl blue were prepared with bi-distilled miliQ water filtered with MiliQ filters. Ethanol 96% Panreac was used.

2.2 Devices design and fabrication

A 10x10 channel array was designed in CAD. The array is defined by 2 mm long channels separated by each other horizontally by 5 mm and by 3 mm vertically (fig. 3)

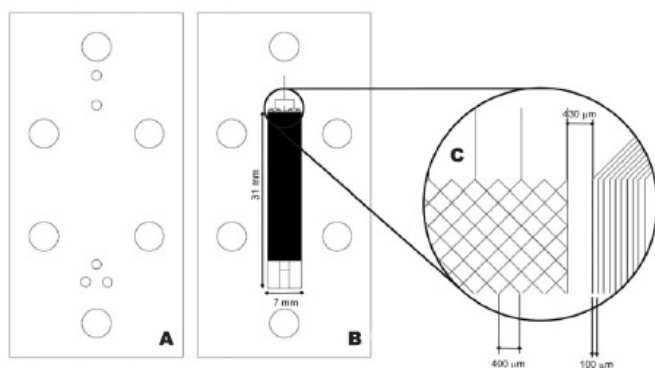


Fig 2. μ -FFE device layers design A) Inlet and outlet layer B) Diamond shaped microfluidic structure layer C) Diamond shaped microfluidic structure detail

For the μ -FFE, a separation chamber (31 mm long, 7 mm wide), with diamond shaped post of 400 μ m edge length was designed. The 430 μ m dielectric barrier, separates the separation chamber (2 inlets and 3 outlets) from de electrode channels. The electrode channels are 1 mm wide, defined as a 10 channels array separated by 100 μ m from each other, and have 1 inlet and 1 outlet each. 6 mm diameter holes were designed for screw insertion. The CircuitCAM software was used to adapt CAD designs to the LPKF ProtoLaser 100 (LPKF Germany) control software (BoardMaster). In BoardMaster cutting tools were created tools were attributed to etch structured of the design. Low temperature co-fired ceramics 951 AX (DuPont) were cut, placed between 2 stell plates and laminated by thermo compression, in an uniaxial hydraulic press (Talleres Francisco Camps S.A., Granollers, Espanha) at 23-27bar and 100 $^{\circ}$ C, for 5 to 30 s, depending on layer structure. Finally, the laminated device was burnt-out in air atmosphere in a Carbolite CBCWF11/23P16 furnace. The laminates were (10 $^{\circ}$ C/min)

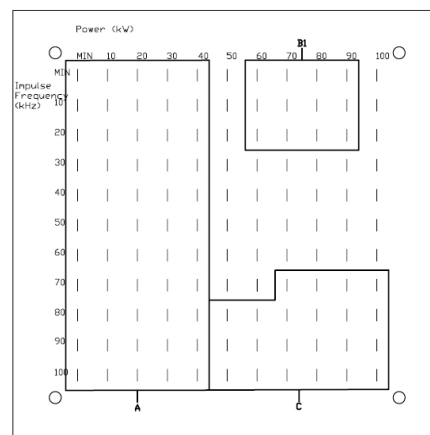


Fig 3. Channel array with zones

burnt-out to 350 $^{\circ}$ C, followed by sintering at 850 $^{\circ}$ C (also achieved at 10 $^{\circ}$ C/min). After burning, LTCC devices were inspected with a KLA Tencor P-15 profilometer, and a Minaron MTV-88-EX CCD camera

A 3,5 mm thick PMMA (Pexiglass) substrate was cut with a CNC Protomat C100 milling machine to 90 mm x 50 mm and 6, 6 mm diameter holes for screw insertion were drilled.

The PDMS (Dow Coring Sylgard 184) 1,5 mm layer was fabricated by mixing pre-polymer with curing agent in a 10:1 ratio. The mixture was vigorously mixed for 5 minutes to improve homogeneity and left in vacuum desiccator until total elimination of air bubbles. The mixture was poured onto Petri dish and cured for 15 minutes, at 120 $^{\circ}$ C. PDMS was then cut and lifted off using ethanol.

The PDMS layer was assembled between the LTCC and PMMA substrates and pressed using conventional screw and nuts inserted in the 6 mm diameter holes.

Microfluidic connectors were epoxy glued to the inlets and outlets of the device. Tygon 0,8 mm i.d tubing (Bioblock) was

inserted in the microfluidic connectors in order to function as interface with the exterior.

Luer lock system with 0,3 mm i.d teflon tubing were attached to Hamilton Gastight syringes

One of the syringes previously filled with Wood's alloy (Goodfellow) was connected to the electrode inlets. The device and syringe were placed in an oven at 150 °C, and the syringe was manually pressure, until the liquid alloy filled the channel. Conventional clamps were used to seal the tygon tubing, closing the fluidic path of the alloy, and the device was let to cool down to room temperature

Syringes filled with phenol red 1M, and methyl blue, were assembled in 2 TSE 540060-OEM syringe pump, and connected to the inlet of the μ -FFE

3 Results

3.1 Channel array

The channel array will be referred to as in typical a_{ij} matrix nomenclature. Aside from power and impulse frequency, other cutting parameters were maintained constant (fig. 4).

After inspection of the channel array with a CCD camera, 3 zones were identified. In the zone C (Fig 3), the channels fabricated were very irregular. For this reason, this area was discarded for profilometer inspection. In zone A of the channel array, only in a_{14} and a_{24} channel were fabricated. According to the manufacture, this is caused by the instability of the ProtoLaser, when working at less that 50 % of the it's total power.

		Power (kW)					
		50	60	70	80	90	100
Impulse Frequency (kHz)	10	115	135	160	155	170	170
	20	85	110	155	160	180	160
	30	60	90	140	120	125	130
	40	70	75	105	110	110	105
	50	45	80	100	160	95	85
	60	45	105	95	130	75	75
	70	45	80	-	-	-	-

Tab.1. Channel array width

		Power (kW)					
		50	60	70	80	90	100
Impulse Frequency (kHz)	10	70	90	100	100	100	100
	20	50	90	100	100	95	95
	30	40	70	90	95	95	90
	40	40	45	70	80	75	65
	50	25	45	50	100	55	45
	60	25	85	70	95	35	35
	70	25	55	-	-	-	-

Tab. 2 Channel array width

For this reason, this area was also discarded for profilometer inspection.

In zone B, a sub zone (B1) was identified just after the cutting process. The LTCC layers in this zone were completely cut, originating channels of 254 μ m in unfired state. The obtained results for channel depth (100 μ m) reflect the maximum depth measured by the profilometer

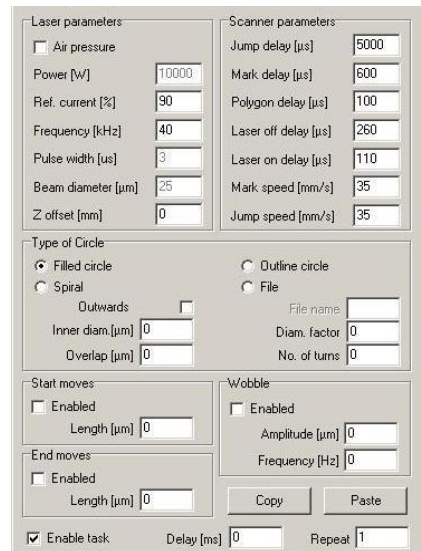


Fig. 4. Laser cutting parameters

For a constant power, the increase of the impulse frequency, originates the reduction of the dimensions of the channels depth and width (tab 1 and tab 2). Being P the continuous Power of the laser, W , work and t , time, we have

$$P = \frac{W}{t} \quad (3)$$

since

$$v = \frac{1}{t} \quad (4)$$

with v being the frequency, we get

$$P = w \cdot v \quad (5)$$

Thus increasing the impulse frequency reduces the energy of each impulse thus reducing the dimensions of the channels

For a constant impulse frequency, the increase of power, originates the increase of wide and depth of the channels, reaching a maximum value between 70 and 80 kW, From this power on the a increase in channel wide, and a decrease in channel depth. Probably the heat generated by the laser contact with ceramics, with these laser parameters, reaches melting temperature of glass. Thus part of the energy is used in the cutting process of alumina and part in the melting process of glass.

3.2 μ -FFE

Using the channel array results, and taking into account that LTCC substrates suffer 15% shrinkage in the x-y plane during sinterization the μ -FFE could be designed with micrometric accuracy. In order to use a_{49} parameter (110 μm width and 75 μm depth channels), the μ -FFE was designed to have a 250 μm dielectric layer. However, conventional thermo lamination, originated

deformation of the microfluidic diamond shaped structures. These deformations could be minimized by diminution of lamination time, this could originate delamination problems. In the future a low pressure lamination [11] should be employed in order eliminate microfluidic structure deformation.

Using a diamond shaped fluidic LTCC structure, allowed the use of PDMS as o-ring in a LTCC-PDMS-PMMA gasket, since the structures between the channels supported the PDMS. However the entrance of the liquids through the inlet channels were some times obstructed, do to PDMS pressure. This is due to the entrance of liquids to be perpendicular to the channels and implicates, that inlets position must be modified in order to solve this problem.

Fluidic tests proved the effectiveness of LTCC-PDMS-PMMA gasket like system, the device separation ability was not tested do to lack of time. It seems however that, the separation of non-fluorescent dyes will be difficult to monitor due to low contrast achieved between coloured dyes and the blue coloured LTCC.

4 Conclusions

A study of the influence of frequency of impulse and power in the channel fabrication in LTCC by laser ablation was conducted. The data obtained is a useful and precise tool, for the design of microfluidic structures. Furthermore, it allowed for the first time the construction of microfluidic structures in the range of tenths of μm , a 25 μm of depth and 45 μm channel

as the smallest microfluidic structure fabricated.

The possibility of fabricating microfluidic complex structures with the use of a LTCC-PDMS-PMMA gasket like system proved to be a simple, modular method to seal LTCC device, allowing the use of optical detection systems.

Finally the information obtained was used to fabricate an electrostatic induction electric field generation approach μ -FFE device, with a complex diamante shaped microfluidic structure. The use of thermo compression lamination system showed not to be appropriate for the use with complex microfluidic motives. In the future a no pressure lamination should be employed in order eliminate microfluidic structure deformation. Duo to lack of time the separation ability of the device was not tested. This should be the next step of the project.

5. References

- [1] A. Manz, N. Graber, H. M. Widmer, Miniaturized total chemical analysis systems: A novel concept for chemical sensing, *Sensors and Actuators B* 1 (1990) 244-248.
- [2] Reyes, D. Iossifidis, P. Aroux, A. Manz, *Micro Total Analysis Systems*. 1. Introduction, Theory, and Technology, *Analytical Chemistry* 74 (2002) 2623-2636.
- [3] M. Gongora-Rubio, P. Espinoza-Vallejos, L. Sola-Laguna, J. Santiago-Avilés, Overview of low temperature co-fired ceramics tape technology for meso-system technology (MsST), *Sensors and Actuators A* 89 (2001) 222-241.
- [4] D. Kohlheyer, J. Eijkel, A. Van den Berg, R. Schasfoort, Miniaturizing free-flow electrophoresis - a critical review, *Electrophoresis* 29 (2008) 977-993.
- [5] J. Bauer, (Ed.), Special Issue on Free-Flow Electrophoresis, *Electrophoresis* 19 (1998) 1057-1235.
- [6] D.E. Raymond, A. Manz, H. Widmer, Continuous Sample Pretreatment Using a Free-Flow Electrophoresis Device Integrated onto a Silicon Chip, *Analytical Chemistry* 66 (1994) 2858-2865.
- [7] B.R. Fonslow, V.H. Barocas, M.T. Bowser, Using Channel Depth To Isolate and Control Flow in a Micro Free-Flow Electrophoresis Device, *Analytical Chemistry* 78 (2006) 5369-5374.
- [8] C.X. Zhang, A. Manz, High-Speed Free-Flow Electrophoresis on Chip, *Analytical Chemistry* 75 (2003) 5759-5766.
- [9] D. Kohlheyer, G. A. J. Besselink, S. Schlautmann., R. B. M. Schasfoort, Free-flow zone electrophoresis and isoelectric focusing using a microfabricated glass device with ion permeable membranes, *Lab Chip* 6 (2006) 374-380.
- [10] D. Janasek, M. Schilling, A. Manz, J. Franzke, Electrostatic induction of the electric field into free-flow electrophoresis devices, *Lab Chip* 6 (2006) 710-713.
- [11] M.R. Gongora-Rubio, Z. Rocha, N. Ibañez-García, N.A. Oliveira, J.R Matos, Low Temperature and Pressure Lamination of LTCC Tapes For Meso-Systems, in: IMAPS Conference and Exhibition on Ceramic Interconnect Technology, 2004 Proceedings in CD-ROM.