Available online at www.sciencedirect.com
SCIENCE DIRECT
Sensors and Actuators A 127 (2006) 207–215
SENSORS AND ACTUATORS A PHYSICAL
www.elsevier.com/locate/sna

An integrated system for on-chip temperature gradient interaction chromatography

Chi-Yuan Shih *, Yang Chen, Wei Li, Jun Xie, Qing He, Yu-Chong Tai

M/C 136-93, Department of Electrical Engineering, California Institute of Technology, 1200 E California Bl, Pasadena, CA 91125, USA

Received 23 February 2005; received in revised form 20 July 2005; accepted 26 August 2005
Available online 29 September 2005

Abstract

This paper reports the first integrated MEMS high performance liquid chromatography (HPLC) chip that consists of a parylene high-pressure liquid chromatography (LC) column, an electrochemical sensor, a resistive heater and a thermal-isolation structure for on-chip temperature gradient interaction chromatography (TGIC) application. Two sets of devices for different chromatography criteria were fabricated. The separation column was 8-mm long, 100-μm wide, 25-μm high and was packed with 5-μm sized, C18-coated beads using conventional slurry-packing technique. A novel parylene-enhanced air-gap thermal isolation technology was used to reduce heater power consumption by 58% and to reduce temperature rise in the off-column area by 67%. To test the chromatography performance of the fabricated system, a mixture of derivatized amino acids was chosen for separation. A temporal temperature gradient scanning from 25 °C to 65 °C with a ramping rate of 3.6 °C/min was applied to the column during separation. Successful chromatographic separation of derivatized amino acids was obtained on the chip. Compared with conventional temperature gradient HPLC system which incorporates "macro oven" to generate temporal temperature gradient on the column, our chip's thermal performance, i.e., power consumption and thermal response, is greatly improved without sacrificing chromatography quality.

Keywords: Temperature gradient; Lab-on-a-chip; Parylene; Amino acid; Thermal isolation; Electrochemical sensing

1. Introduction

HPLC is one of the most powerful analytical tools in the field of separation science. In the past decade there has been a growing interest and demand among industry and academia in developing a new generation of HPLC system that can provide better separation efficiency, higher sensitivity, shorter analysis time and lower cost [1–3]. In fact, the logic trend of modern HPLC instrumentation has been the miniaturization of system components. For example, the reduction of separation column inner diameter (i.d.) increases separation sensitivity which is especially critical for modern proteomics where in many cases only meager amount of sample molecules is available for analysis [4]; smaller column i.d. reduces mobile phase solvent consumption and therefore means lower cost for each separation task and is environmentally-friendly; smaller stationary-phase beads result in higher separation efficiency [4]; the miniaturization of solvent pumps makes HPLC system portable and therefore enables many applications such as point-of-use HPLC analysis or hand-held system for personal heath diagnostics [5].

It is foreseeable that MEMS/lab-on-a-chip technology, given its strength and flexibility in micro fabrication, should be a powerful tool to achieve HPLC system miniaturization. However, despite the abundant publications on chip-based capillary electrophoresis (CE), the on-chip pressure-driven HPLC is relatively under-exploited [6–10]. One main reason for that is the lack of straightforward techniques to integrate various components of a complete HPLC system on a single chip such as the bead-packed separation column, analyte sensor, solvent pump and others. Only recently chip-based integrated HPLC and LC-ESI systems were reported [6–13] and successfully used for ion chromatography [11] and proteomics [12]. The long-term goal is to incorporate a broader range of separation functionality into a single chip and demonstrate that chip-based HPLC is as good as, if not superior to, conventional HPLC in terms of chromatography performance.

Reversed-phase LC is one of the most popular separation strategies utilized in proteomics or macromolecule separations

* Corresponding author. Tel.: +1 626 395 2227; fax: +1 626 584 9104.
E-mail address: cyshih@caltech.edu (C.Y. Shih).

0924-4247/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.sna.2005.08.013

[12]. However, for miniaturized reversed-phase LC systems, many challenging factors still keep them away from practical use. For example, mobile phase pumps need to maintain low and smoothly changing flow rates (~100 nL/min) which contribute to the right solvent gradient profile for separation. Also, thorough mixing among mobile phase solvents needs to be done in the micro scale within a relatively short time [12]. It is therefore more difficult for chip-based system to generate high quality solvent gradient, which in turn sacrifices chromatography performance. On the other hand, it was found that temporal temperature gradient (i.e., changing LC column's temperature as a function of time) could be as effective as solvent gradient. In other words, for exothermic interaction between the analyte molecules and the stationary phase of separation column, low temperature elution is similar to polar solvent elution and high temperature elution is similar to non-polar solvent elution. The temperature (T) dependence of analyte retention factor (k) can be expressed as [14]:

$$\ln k = -\frac{\Delta H_0}{RT} + \frac{\Delta S_0}{R} + \ln \beta$$

where ΔH_0, ΔS_0 are the standard enthalpy and entropy changes of analyte phase transfer from the mobile phase to the stationary phase; R, the gas constant; β, the phase ratio. Using temperature gradient to achieve or improve analyte separation is categorized as temperature gradient interaction chromatography (TGIC) [14–21]. More importantly, compared with solvent gradient, temperature gradient is much easier to carry out on miniaturized HPLC system. In fact, chip-based TGIC system is extremely attractive because of the fast and accurate on-chip temperature control by MEMS technology as demonstrated in polymerase chain reaction (PCR) [22,23]. As far as we know, chip-based TGIC has never been demonstrated. In this paper, we then report an integrated TGIC system with on-chip bead-packed separation column, electrochemical analyte sensor, resistive heater and parylene-enhanced thermal isolation structure. Using the developed system, we have successfully demonstrated the first on-chip TGIC of amino acids.

2. Experimental

2.1. Chip design and fabrication

The designed MEMS TGIC chip contains components including: an HPLC column which can stand column inner pressure of at least 200 psi without leaking or breaking during bead-packing and chromatography procedures; an integrated resistive heater which provides on-chip temporal temperature gradient as the elution mechanism; an electrochemical sensor for on-chip analyte detection; an integrated thermal isolation structure to the column for the precise column temperature control and the reduction of heater power consumption. Two versions of devices were fabricated to satisfy different chromatography criteria. Version I device was designed to operate at lower power consumption (71 mW) and lower chromatographic back pressure (180 psi). Version II device was designed to operate at higher power consumption (400 mW) and higher chromatographic back pressure (600 psi).

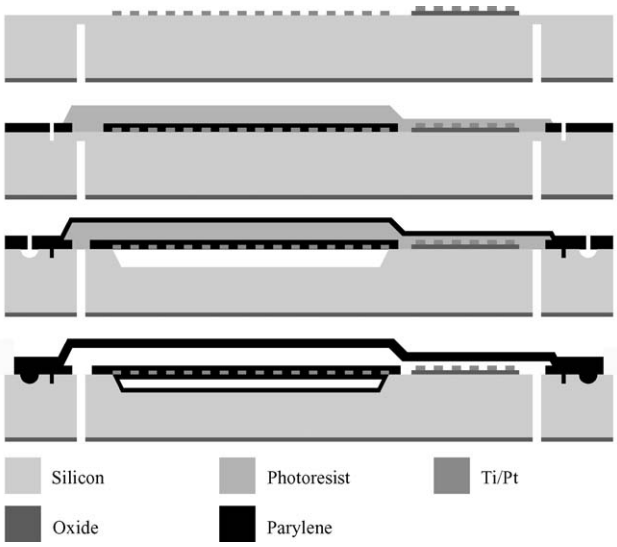

Fig. 1. Device process flow for Version I MEMS TGIC chip.

2.1.1. Version I

In order to minimize power consumption for the temperature gradient operation, column with imbedded heater was made freestanding as shown in the process flow (Fig. 1). The integrated electrochemical sensor was cooled by silicon substrate to maintain steady temperature during temperature gradient operation. Fabrication process started with growing 1.1-μm thick SiO_2 layer on both sides of 4-in. silicon wafers (thickness: 525 ± 25 μm) by thermal oxidation. Front side oxide was then patterned with buffer HF (Transene, Danvers, MA). 300 Å-titanium/2000 Å-platinum/1000 Å-gold was e-beam evaporated on wafer front side. Backside oxide was then patterned with buffer HF to define the 100-μm diameter liquid access holes. Without removing photoresist on top of the backside oxide, DRIE (deep reactive ion etching) with the standard Bosch process was used to etch backside silicon for 500 μm. Gold was then patterned with wet etchant (Transene, Danvers, MA). Here, gold was used to provide an interface for easy gold-wire bonding. Ti/Pt was then patterned with wet etchants to define the resistive heater and electrochemical sensor. 3.5-μm thick first parylene-C layer (Specialty Coating Systems, Indianapolis, IN) was deposited and patterned with oxygen plasma on the wafer front side as the column bottom wall. Twenty-five micrometers photoresist AZ4620 (Clariant, Somerville, NJ) was deposited and patterned on wafer front side to form a two-level photoresist structure using a double-exposure method [11]: one full exposure (first mask) and one partial exposure (second mask) were performed prior to developing the photoresist; beads filters and electrochemical sensing areas were partially exposed so to leave only 4 μm photoresist after developing; the unexposed photoresist formed the 25-μm thick sacrificial layer for the liquid chromatography columns and inlet/outlet chambers; photoresist was then developed and a 100 °C-3 h baking was carried out to avoid photoresist bubbling during latter thermal