AUTHOR QUERY FORM

ELSEVIER	**Journal:** CHROMA **Article Number:** 351730	**Please e-mail or fax your responses and any corrections to:** **E-mail:** corrections.esnl@elsevier.thomsondigital.com **Fax:** +353 6170 9272

Dear Author,

Please check your proof carefully and mark all corrections at the appropriate place in the proof (e.g., by using on-screen annotation in the PDF file) or compile them in a separate list.

For correction or revision of any artwork, please consult http://www.elsevier.com/artworkinstructions.

Any queries or remarks that have arisen during the processing of your manuscript are listed below and highlighted by flags in the proof. Click on the 'Q' link to go to the location in the proof.

Location in article	Query / Remark: click on the Q link to go Please insert your reply or correction at the corresponding line in the proof
Q1	Reference(s) given here were noted in the reference list but are missing from the text – please position each reference in the text or delete it from the list. Uncited reference: This section comprises reference that occur in the reference list but not in the body of the text. Please position the reference in the text or, alternatively, delete it. Any reference not dealt with will be retained in this section.

Thank you for your assistance.

Journal of Chromatography A, xxx (2010) xxx–xxx

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Short communication

Integration of ground aerogel particles as chromatographic stationary phase into microchip

Attila Gaspar*, Andrea Nagy, Istvan Lazar

Department of Inorganic and Analytical Chemistry, University of Debrecen, P.O. Box 21, H-4010 Debrecen, Hungary

ARTICLE INFO

Article history:
Received 2 November 2010
Received in revised form
18 December 2010
Accepted 20 December 2010
Available online xxx

Keywords:
Aerogel
Polydimethylsiloxane (PDMS) microchip
Chromatographic packing

ABSTRACT

C16 modified and ground monolithic silica aerogel particles in submicrometer size, as a new type of stationary phase was prepared and integrated in ~~polydimetilsiloxane~~ (PDMS) microchip. The aerogel particles were packed into the microfluidic channel using a simple procedure, which does not require any special frit or fabrication step to retain the particles. The subnanoliter volume of samples can be transported through the porous, short length of packing with low pressure (<3 bar). Food dyes as test components could be separated using low pressure within 6 s. A 50-fold preconcentration could be achieved by retaining 100 nL volume of sample on the packing and elution with methanol.

1. Introduction

Porous bed is the heart of chromatography since the most essential feature of the chromatographic process is the percolation of the liquid or gas (mobile phase) through the stationary phase [1]. To achieve high peak resolution, the contact surface area between the stationary and mobile phases, as well as the permeable intraparticle pore space should be as large as possible. That can be reached either by using small chromatographic particles or porous monolithic columns. However, the hydraulic resistance of the bed to the stream of mobile phase should be moderate. The best chromatographic stationary phases have a high volume of through pores and low hydraulic resistance. One of the main trends in chromatography is to develop new monolithic stationary phases [1–3], because those seem to meet quite well the mentioned demands. However, there are some disadvantages of the monolith columns, too: possible wall effects [1,4], problems in reproducibility and characterization of the manufactured columns.

Monolithic silica aerogels differ from traditional silica gels and from the chromatographic monoliths significantly both in structure and porosity and can be created by a special supercritical drying technology [5]. Silica aerogels hold records for the lowest bulk density of any solid materials (as low as 0.003–0.5 g/cm^3), the highest specific surface area of any monolithic materials (up to 1600 m^2/g) and they show unique characteristics in mechanical, acoustical, thermal, optical and many other fields [6]. Contrary to their extremely high internal porosity (90–95 v/v%), monolithic silica aerogels have not yet been used in any kind of chromatographic separations as stationary phases.

Nowadays there is a high demand for miniaturized chromatographic techniques that would provide the same very versatile applications that have been found in liquid chromatography (LC). However, only a few chip-based chromatographic systems have been reported so far as compared to chip-based capillary electrophoretic (CE) devices, which is mainly due to technical problems inherent in the former [7,8]. Most microfluidic devices are based on open tubular flow designs and only a few involve packed-bed chips due to the difficulty in preparing frits [9,10]. Recently, this problem has been excellently addressed [11], however, the high-tech manufacturing of the three-laminated polyimide layers chip (use of laser, micromachined frit) is not possible for the large majority of the researchers.

In our recent work we described the fabrication of a PDMS-based microfluidic chip containing reversed-phase silica beads without the use of frits or other barriers and different retaining and stabilizing effects appearing in the packed channel have been observed [12]. In this work we demonstrate a new procedure to pack submicrometer particles into a part of the microfluidic channel, and for the first time to prepare and test ground silica arogel particles as stationary phase for low-pressure chromatography.

2. Experimental

2.1. Preparation of C16-functionalized monolithic silica aerogel and obtaining submicrometer size particles from it

A solution of tetramethoxysilane (TMOS, 2.25 ml) and hexadecyl trimethoxysilane (HDTMOS 0.25~~0~~ ml) in methanol (9.90 ml) was

* Corresponding author. Fax: +36 52 489667.
E-mail addresses: gaspara@tigris.unideb.hu, ~~gaspara@tigris.klte.hu~~ (A. Gaspar).

0021-9673/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2010.12.091

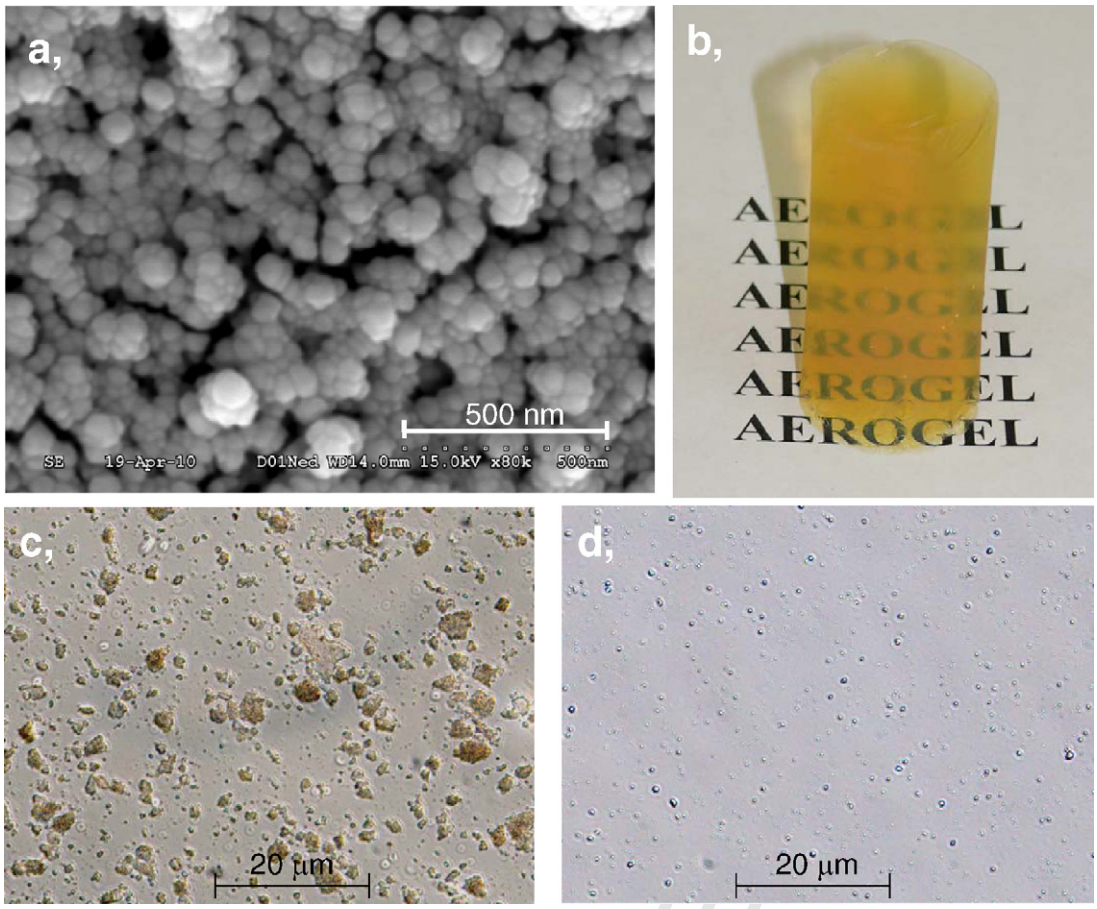

Fig. 1. (a) SEM image of the porous structure of a monolitic silica aerogel. (b) Photo of a block of C16 silica aerogel (0.091 g/cm^3). Aerogel particles powdered in a ball mill and suspended in methanol (c) and the clear liquid above the suspension after 20 min sedimentation (d).

stirred well with a dimethyl formamide (19.8 ml) solution of aqueous ammonia solution (3.30 ml, 25 m/m%-os) and deionized water (2.50 ml) then poured into a tubular plastic mold, sealed and let to stand at room temperature for two days. The alcogel monolith was transfered to a 500 ml volume glass container and soaked first in methanol, then in methanol–acetone mixtures and in acetone, for at least one day in each solvent. The alcogel was then transfered into a 1.4 dm^3 volume supercritical CO_2 dryer under acetone. After sealing the extractor body, acetone was drained. The remaining acetone content of the alcogel was then replaced with liquid carbon dioxide in a slow stream of liquid carbon dioxide at room temperature in 18 h. After 2 h of conditioning at 80 °C the pressure was slowly released in a 4 h period of time. The density of the monolith proved to be 0.091 g/cm^3. The porous structure and a block of the prepared C16 monolithic silica aerogel are shown in Fig. 1a and b, respectively.

About 1 cm^3 volume of the obtained aerogel was powdered in a ball mill for 10 min, then stirred with 1 mL methanol in a Eppendorf tube with a vortex mixer (Fig. 1c). After 10 min of sedimentation the uppermost quarter of liquid layer was sucked to use for the preparation of the chromatographic packing (Fig. 1d).

2.2. Microchip fabrication

The PDMS chips were prepared by using a mold created by soft photolithography [13]. The pattern consisting of standard cross-T type channel of 100 μm wide was printed as a high resolution (10,000 dpi) photomask. Close to the crossing of channels, 10 μm wide line was drawn (Fig. 2a) to create a bottleneck in the channel (starting point to retain particles in the channel). Negative type photoresist (SU-8 2025, Microchem, Newton, MA) was spin-coated onto a 3" silicon wafer at 3000 rpm for 30 s to a thickness of 30 μm. The PDMS chip was fabricated by cast molding of a 10:1 mixture of PDMS oligomer and cross-linking agent (Sylgard 184, Dow Corning, Midland, MI). The PDMS chip was sealed onto a glass slide of 1.2 mm thickness after oxygen plasma treatment (PDC-32G Harrick) (Fig. 2b).

2.3. Microscopy and image analysis

To observe the movement of liquids in the microchip channels and to test the chromatographic characteristics of the packing, food dyes (FD&C blue#1 and FD&C yellow#5, all from McCormick&Co., Inc., MD) were injected and transported in the chip using a low-rate peristaltic pump (IPC, Ismatec). The movement of the plugs was monitored by using an inverted microscope (Axio Observer A1, Zeiss) equipped with a high speed CCD camera. SEM images were taken on a Hitachi S-4300 CFE instrument.

2.4. Chromatographic separation in chip

The samples (0.5–100 μL) were sucked into the peristaltic pump tubing (ID: 0.25 mm), which was initially filled with liquid (water or eluent). This sample was split in the junction and a small volume of the original sample was manipulated into the separation channel (approximately 0.5–100 nL). The sample injected at the sample inlet port (port I in Fig. 2b) flows into the other three channels with different flow rates depending on the hydraulic resistance of each channel. Because the hydraulic resistance in the separation channel of the used chip is estimated to be approximately one

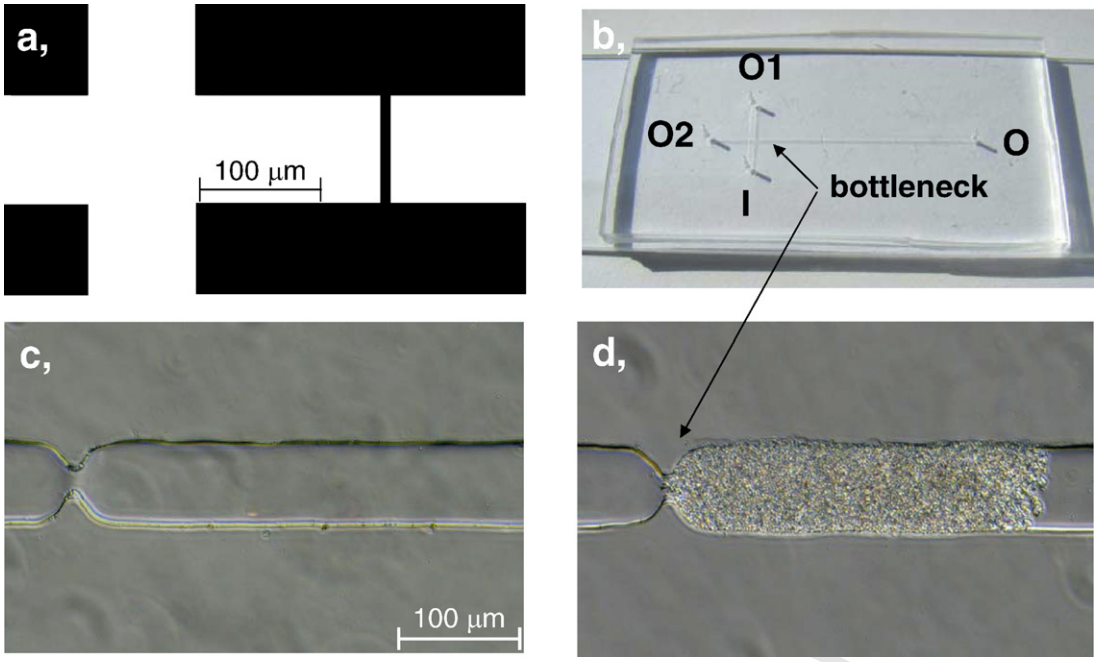

Fig. 2. (a) Part of the lithographic mask pattern about the crossing of channels and the 4 μm wide line positioned close to the crossing to create a bottleneck in the chip (starting point to retain particles in the channel). (b) Photo of the microchip (I: sample inlet, O: separation outlet, O1 and O2 are outlet reservoirs. The suspension of particles is pumped from O during the packing, the sample solutions to be separated or preconcentrated are injected from I). Optical micrographs of the fluid channel with the frit-like bottleneck (c) and the created packing of ground silica aerogel particles (particles were pumped from O port (d)).

thousand times higher (that is the flow rate is one thousand times smaller) than in the other channels, when one microliter of sample is injected into the chip with the peristaltic pump, only about one nanoliter is injected in the separation channel; the majority of the sample solution flows to the waste outlet reservoirs O1 and O2 (ESM Video 1). This simple hydrodynamic injection of subnanoliter volume of samples was characterized in our earlier work [12,14].

The packing was prepared daily, and it was preconditioned with 10 min washing with methanol and 5 min with the mobile phase before chromatographic separation/preconcentration.

3. Results and discussion

3.1. Packing

In order to create a compact, homogeneous packing, a very dilute suspension of little particles was needed. Therefore, ground aerogel particles were dispersed in methanol, homogenized and allowed to be sedimented for 20 min. After that time the clear supernatant layer, which contained the particles of 0.2–1 μm diameter, was sucked out from above the dense sediment and used for packing (Fig. 1d). A few microliters of solution sucked from the clear liquid layer was injected into the chip through the port O (outlet of the separation channel of the chip) and transported toward the bottleneck. At the bottleneck not only the width of the channel is shortened to about 20 μm, but also the height of the channel is reduced from 30 μm to about 5 μm ("3D bottleneck"). The liquid could flow through the bottleneck without remarkable hydraulic resistance (ESM Video 2), but the particles were retained. The first aerogel particles retained around the bottleneck, and then the newly arrived particles adhere smoothly to the packing; increasing its length continuously. The first particles acted as keystones, blocking the other particles. Earlier experiments demonstrated that it was only necessary to taper a rigid capillary [9] or flexible microfluidic channel [12] to approximately 10 μm (inner diameter) to achieve the *keystone effect* [9] and to retain 3 μm sized particles. It was observed that at the bottleneck, the particles were compressed higher and higher resulting in a homogeneous, compact and dense packing as shown in Fig. 2c and d, and ESM Video 3. Finally, the methanol was washed out by water.

Some phenomena regarding the stability of the packing prepared from conventional chromatographic beads were observed. The hard particles adjacent to the wall deform the soft PDMS and partially penetrate into the channel wall which acts as anchors for the packing (anchor effect) [12]. When the pumping pressure (about 1–2 bar) was applied to compress the packing, the wall of the channel was deformed (extended). During this period the particles fill the enlarged volume of the channel and then the channel shrinks when the pressure is released thereby forming a continuous strain around the packing. The particles of the packing are compressed by the forces of the elastic strains acting perpendicularly from the wall toward the middle of the channel (clamping effect) [12].

3.2. Chromatographic test

The C16 modified silica aerogel particles are expected to have similar chromatographic features as the C18 conventional chromatographic beads. The blue food dye can be completely retained on the aerogel packing in aqueous solution. The retained component was not immobilized by washing with water, but it could be immediately eluted with methanol. Therefore this component could be effectively concentrated on the packing. When 100 nL volume of diluted dye sample was pumped toward the packing (about 300 s), the components were completely retained and concentrated in the first 250 μm length of the packing. The subsequent methanolic washing eluted the components merely in a 2 nL volume, that is, about 50× concentration could be achieved. This concentration efficiency can be further increased if larger sample volume is used (nevertheless, a few microliter volume of sample generally is available for an analysis). The overall extraction capacity of the packing related to the blue component was calculated to be 7.5×10^{-11} mol/μm packing (cross-section area of the packing: 0.0035 mm^2). While in aqueous solution the blue food dye was completely retained, the yellow dye was passed through the pack-

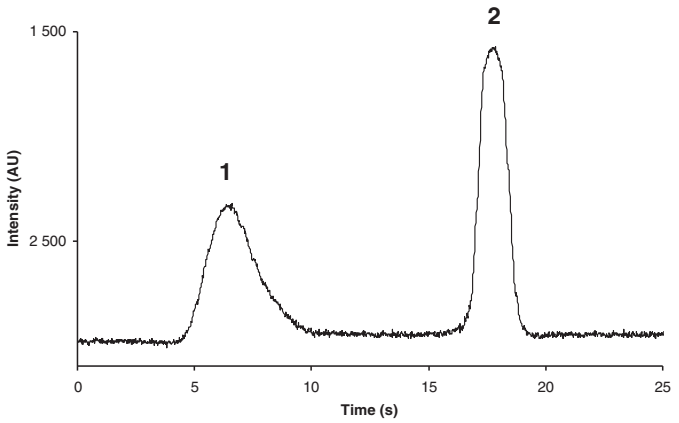

Fig. 3. Separation of food dyes (1: yellow, 2: blue) in chip packed with ground C16 modified silica aerogel particles. The water mobile phase was changed to methanol when sample plug was reached the packing. The flow rate in the separation channel was 0.2 nL/s.

ing. Changing the mobile phase to methanol, the blue component was washed out (Fig. 3).

The two dye components could be separated under isocratic condition, too. Using 35% methanol:water as mobile phase the dyes were eluted one after the other. A chromatographic packing of 1 mm length was suitable for the separation of 1 nL volume of the dye mixture (Fig. 4). Using the maximal pumping pressure with the peristaltic pump (about 3 bars), extremely fast separation has been achieved.

High efficiency of separation can be obtained within a short separation length only if the length of the injected sample plug (l_{inj}) or the detector observation length (l_{det}) can be shortened to a minimum. l_{det} could be kept minimal due to the microscopic detection in a single point (pixel) based on measuring the intensity of colors (RGB) at any part of the channel.

In a hunt for the fastest chromatographic separation 0.5 mm length of packing (L), 1 nL volume of sample (l_{inj}: 250 μm) and the maximal pressure of the peristaltic pump (3 bar) was applied (ESM Video 4). When right after the packing was detected almost baseline separation was achieved within 8 s (Fig. 5c). Since the thickness of the packing in the transparent PDMS chip is only 35 μm, the dyes could be monitored and detected even through the packing (Figs. 4a and b and 5a and b). While an only 200 μm travelling through the packing could not result in complete separation yet, 300 μm length provided baseline separation of the dyes within 6 s. The detection beyond the packing gives worse separation due to the dispersion of the components (Fig. 5d).

4. Conclusion

This is the first case reported so far when a silica aerogel was used as a chromatographic stationary phase in a microchip. The great advantage of ground monolithic aerogel particles over other phases is the very high inner porosity ($\varepsilon > 0.90–0.95$) and the homogenous distribution of wide 10–100 nm mesopores which form a dynamically readily accessible open pore system with a large number of throupores. In comparison, the monolithic columns have approx. 2–3 μm sized open channels and 10–13 nm mesopores within the porons (with an overall porosity of $\varepsilon = 0.85$), while conventional silica-based stationary phases are characterized with 6–15 nm mesopores and moderate porosity ($\varepsilon = 0.62$) [16]. Although the solid macroscopic skeleton of the aerogel collapsed when it got contact with any liquid (water), the average size of the particles obtained after grinding and suspending was about 0.2–1 μm.

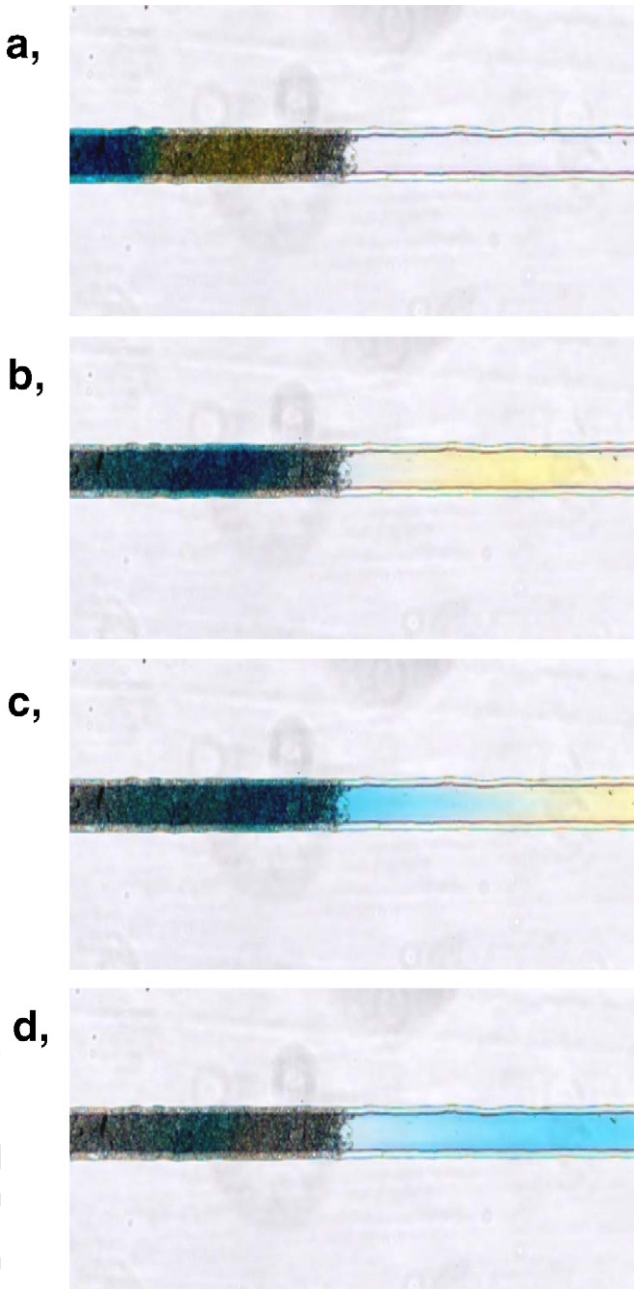

Fig. 4. Optical micrographs of the silica aerogel packing during separation of food dye components using 35% methanol:water as mobile phase. The flow rate in the separation channel was 2 nL/s (maximal rate attainable by peristaltic pump). (Time between (a) and (d) is 8 s.)

The aerogel particles were packed into the microfluidic channel using a simple procedure, which does not require any special frit to retain the particles. The bottleneck in the channel can be easily created at any part of the microfluidic chip. The simplicity of replication of the PDMS chips and the minimal consumption of the conventional packing particles (some tens of nanograms for a 10 mm length of packing) makes the chips inexpensive and disposable. Since reversed-phase silica particles are widely used as the stationary phase in HPLC and SPE, the described chip-based chromatographic system has great potential in many applications (e.g. preconcentration, purification, separation). Further optimization of the chromatographic separations would be beneficial.

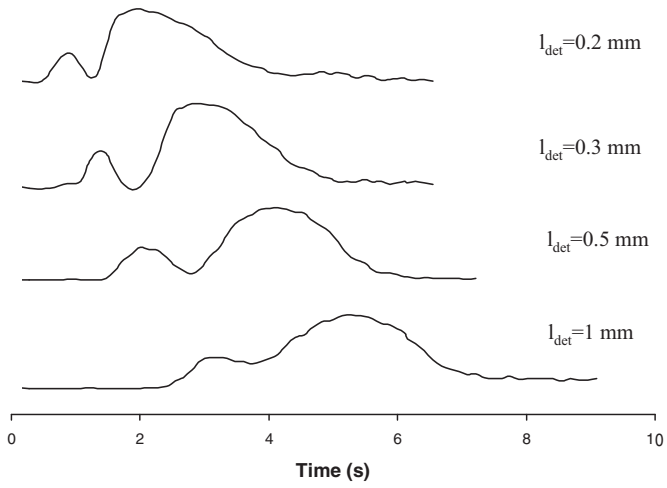

Fig. 5. Separation of food dyes in chip packed with ground C16 modified silica aerogel particles using 35% methanol:water as mobile phase. The color intensity measurement (detection) was carried out at 0.2 mm (a), 0.3 mm (b), 0.5 mm (c) and 1 mm (d) from the front part edge of the packing. The flow rate in the separation channel was 2 nL/s.

Q1 **Uncited reference**

[15].

Acknowledgements

The authors gratefully acknowledge financial support obtained from the National Scientific Research Fund, Hungary (K75286, K76834) and TÁMOP 4.2.1./B-09/1/KONV-2010-0007 project. The authors also would like to express their thank to L. Daróczi (UD) for recording SEM images and H. Bereczki and M. Andrási (UD) for help in preparation of the C16 silica aerogel and the PDMS chips, respectively.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2010.12.091.

References

[1] G. Guiochon, J. Chromatogr. A 1168 (2007) 101.
[2] S. Hjerten, J.L. Liao, R. Zahang, J. Chromatogr. A 473 (1989) 273.
[3] F. Svec, J.M.L. Frechet, Anal. Chem. 64 (1992) 820.
[4] I. Nischang, O. Brueggemann, F. Svec, Anal. Bioanal. Chem. 397 (2010) 953.
[5] S.S. Kistler, Nature 127 (1931) 741.
[6] N. Husing, U. Schubert, Angew. Chem. Int. Ed. 37 (2007) 22.
[7] B. He, N. Tait, F. Regnier, Anal. Chem. 70 (1998) 3790.
[8] A. de Mello, Lab Chip 2 (2002) 48N.
[9] L. Ceriotti, N.F. de Rooij, E. Verpoorte, Anal. Chem. 74 (2002) 639.
[10] C. Ericson, J. Holm, T. Ericson, S. Hjertén, Anal. Chem. 72 (2000) 81.
[11] S. Ehlert, K. Kraiczek, J.A. Mora, G.P. Rozing, U. Tallarek, Anal. Chem. 80 (2008) 5945.
[12] A. Gaspar, M.E. Piyasena, F.A. Gomez, Anal. Chem. 79 (2007) 7906.
[13] D.C. Duffy, J.C. McDonald, O.J.A. Schueller, G.M. Whitesides, Anal. Chem. 70 (1998) 4974.
[14] A. Gaspar, L. Hernandez, S. Stevens, F.A. Gomez, Electrophoresis 29 (2008) 1638.
[15] C.F. Poole, The Essence of Chromatography, Elsevier, Amsterdam, 2003.
[16] A.M. Siouffi, J. Chromatogr. A 1000 (2003) 801.