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High-performance liquid chromatography on glass chips using precisely defined porous polymer monoliths as particle retaining elements

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ABSTRACT

A stable and permanent integration of miniature packed bed separation columns into microfluidic systems is a major issue in nano liquid chromatography. Various approaches like differently shaped retaining elements or the use of key stone effect have been investigated. We show a flexible integration of miniature packed bed separation columns into microfluidic chips utilising common HPLC material achieved by laser-assisted generation of narrow, photopolymerised frits. The generated retaining elements serve as an in- and outlet frits for the columns. An optimised pre-polymeric solution, consisting of butyl acrylates and a porogen, allows a precise fabrication of frit-type structures with lengths of less than 100 μm and the capability to withstand common slurry packing pressures of more than 250 bar. The separation of seven polycyclic aromatic hydrocarbons by pressure-driven, reversed-phase chromatography proves the high quality of the created chromatographic column inside a glass chip. Plate heights down to 2.9 were achieved and extremely fast separations with sub-second peak widths were performed in isocratic and gradient elution modes on very short columns (≤25 mm).

1. Introduction

In past decades, miniaturisation of liquid chromatography has progressed considerably with the aim of low sample and negligible eluent consumption, and easy accessibility to electrospray-mass spectrometry due to the improved performance of the resulting nano-spray. Despite those benefits, adoption of nano liquid chromatography (nano-LC) is slow. In comparison to HPLC as one of the workhorses in analytical laboratories nano-LC had the stigma of being less robust and reliable and not suited for daily routine analysis outside academic laboratories. A major argument against this technique were the high demands considering the system void volumes which ideally have to be eliminated or at least be reduced to preserve the extremely low peak volumes achievable in nano-LC. In this context the challenge of connecting fragile capillary columns with fittings to HPLC systems in a reliable and robust manner is one of the most important issues. An elegant approach to reduce dead volumes is system integration, realised by the application of microsystem technology. This has led to a renaissance of nano-LC in terms of chip-chromatography [1–4], a novel technique which has already found its way into various commercial products [5–8].

Generation of the chromatographic column inside the microfabricated channels is a central aspect for integration of liquid chromatography on chip. The inclusion of common particulate chromatography columns relies on the presence of particle retaining elements. An elegant way to integrate such components inside a micro channel is by generation of a confinement structure during the microstructuring process of the chip. In this context the most common bead trapping approach is tapering of the channel to provoke particle stacking either by use of the key stone effect [9] or by creating channel segments smaller than the bead size [10]. A wide range of differently shaped retaining elements was investigated for this purpose such as multi-channel types (frit-like) [3,11–17] or a confined single channel appearing in form of a weir [10,18,19], a step [7] or a smooth taper [9,20]. Particulate chromatographic beds in microfluidic devices are generated by slurry packing against these structures. However, the operation of such devices incorporating a single retaining element is restricted to unidirectional fluid flow to preserve the stability of the particulate bed. The integration of a second retaining element ensures higher column stability against pressure variances during operation. Additionally, two retaining elements allow operation under reversed flow direction. This has been demonstrated for electroosmotic [10]

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and pressure driven [21] chip-LC. While this approach demonstrates the strength of microsystem technology for integration of separation columns, the need of an additional packing channel, which has to be closed after packing, is a drawback. In addition the column lengths is unalterable. This is a disadvantage, especially in prototyping and method development. Therefore a more flexible method of column integration is desirable.

Besides the integration of restraining structures for stabilisation, the particulate bed can be immobilised after slurry packing. A likewise approach has been reported for the preparation of LC-chips designed for electrochromatogaphic operation. Jindal et al. developed a method to immobilise beads by use of an embedding monolithic material [22]. However, this approach alters the surface chemistry and physical properties of the column material resulting in changes of chromatographic selectivity and hindered mass transfer [23,24]. Another approach was pursued by Gaspar et al. [25] who exploited the elasticity of the polymeric chip material utilised. Thermal treatment after temporary fixing resulted in a stable column. However the chip material is not compatible with higher pressures.

In the work presented, we utilise photopatterned porous polymeric monoliths (PPMs) as flexible retaining elements, accomplishing high-performance liquid chromatography in high-pressure compatible glass chips. PPMs have been employed as particle retaining elements in nano-LC capillary columns [26]. Such porous sections are fabricated by either thermal polymerisation [27,28] or local photopolymerisation of a pre-polymeric mixture [29]. The generated frits cannot be longer than a few millimetres since the columns typically utilised have lengths of several centimetres. Chip chromatography gives the opportunity to investigate the separation properties of very short columns (millimetre scale) which need equally smaller frits (micrometre scale) as retaining elements. Micrometre-sized photopatterning of macroporous structures is difficult in microchannels of planar devices if traditional shadow mask-assisted methods are utilised. This has been subject in a work of Ramsey et al. [30] who photopatterned a single porous segment to retain particles for solid phase enrichment. Although the PPM-frit in this work has not been designed to withstand high pressures in non-confined channels, some important aspects of the photoinduced, mask-assisted preparation of short PPMs are revealed. Essentially, the success of micrometre-sized, local photopatterning is dependent on various factors. Major aspects are the precision of the illumination [31], the transport mechanisms of reactive species in and outside the illuminated section [32] and therefore the timing of the process. Furthermore, the formation of the PPM is notably affected by interactions with the channel walls. This is an important aspect in respect to the high surface to volume ratio of micrometre sized channels [33,34]. Throckmorton et al. [31], Li et al. [35], and Mair et al. [36] report a practical photolithographical resolution limit for the preparation of permeable monoliths of 200 μm and 100 μm, respectively. These mentioned boundaries were gained by the application of narrow chip slides, cooling, and exact mask-alignment. In addition an investigation of the proper illumination conditions was done for the chip and channel layout utilised and the addressed location in the channel, respectively. The application of a narrow laser beam has proven to significantly enhance the lithographic resolution while avoiding most of the mentioned instrumental preconditions of illumination. Aforementioned was shown for the preparation of nanoporous membranes and sieving gels [37–40].

In this publication, we describe a method to precisely define porous polymeric monoliths, utilising laser-assisted photoinitiation. The properties of such locally photopolymerised PPMs are defined by both, the illumination parameters and the pre-polymer solution used. A pre-polymeric mixture based on butyl acrylates and a porogen for the generation of PPMs, introduced by Shepodd et al. [41], was investigated in a separate study. The results can be found in the supplementary information. By applying mask-assisted methods the formulation of the pre-polymeric mixture was optimised in order to yield short and pressure stable porous frits in capillary tubing.

Herein, we demonstrate precise photopolymerisation of macroporous structures in microfluidic channels by a laser scan of the developed pre-polymeric mixture. The procedure allows the build-up of frit-type monoliths at arbitrary channel locations. This technique provides tailor-made column properties of particulate beds, using common HPLC stationary phases.

2. Experimental

2.1. Reagents and materials

Acetic acid, 2-acrylamido-2-methylpropane sulfonic acid, anthracene, 3-aminofluoranthene, 7-amino-4-methylcoumarin, azobisisobutyronitrile, butyl acrylate (BA), 1,3-butanediol diacrylate (BDDA), 2,2-dimethoxy-2-phenylacetophenone (DMPA), fluoranthene, 3-(trimethoxysilyl)propyl methacrylate (z-6030), pyrene and benzo[*a*]anthracene were purchased from Sigma–Aldrich GmbH (Taufkirchen, Germany). Anthracene-9-carbaldehyde was purchased from Merck KGaA (Darmstadt, Germany) and 1,9-benzanthrone from Riedel-de Haën AG (Seelze, Germany). HPLC-grade acetonitrile, ethanol, isopropyl alcohol, and methanol were purchased from CARL ROTH GmbH CO.KG (Karlsruhe, Germany). High-purity water was provided by a TKA Smart2Pure with 18.2 MΩcm (TKA Wasseraufarbeitungssysteme GmbH, Niederelbert, Germany). Fully porous, particulate material ProntoSil 120-3-C18 SH and ProntoPearl 120-2.2-C18 SH was kindly provided by Bischoff Chromatographie Service GmbH (Leonberg, Germany).

Microfluidic chips used for high pressure applications were fabricated by standard techniques (iX-factory GmbH, Dortmund, Germany). Briefly, two glass slides (borosilicate glass, D263t, 0.7 mm) were structured individually and fusion-bonded afterwards. One slide was wet-etched for the integration of two crossing channels (Fig. 1A). The channels were 90 μm at full width and 40 μm in depth (Fig. 1B). A second glass slide was powder-blasted to provide small access holes (nominal diameter of 364 μm at the bottom, 1 mm at the top).

For photopolymerisation experiments glass/polymer sandwich chips, manufactured via rapid prototyping, were utilised. Aforementioned chips were fabricated, following a multi-step liquid-phase lithographic strategy published elsewhere [42]. The microfluidic structure was formed by a photolithographic polymerisation of polyethylene glycol diacrylate (PEG-DA) matrix, which was sandwiched in between two glass slides.

Placing the chip in a mount enabled high-pressure fluidic connections. The mount (macor material) comprises of two screwable parts, a bottom and a top one, to clamp the chip. Sealing of the port intersection between chip and mount was accomplished by compression of O-rings (1.02 mm cord diameter, 0.74 mm inner diameter, Kalrez, Dupont, Germany) embedded in the top part of the mount. Commercially available ferrules (N-123-04, Upchurch Scientific, IDEX Health & Science LLC) and 3-32″ PEEK-screws (Upchurch Scientific, IDEX Health & Science LLC) provided a connection to fused silica tubing (360 μm outer diameter). No mentionable transfer volumes between the capillary and the O-ring connection could be recognised as inner volumes of an O-ring amounted to approximately 500 nL that of a chip port to approximately 800 nL.