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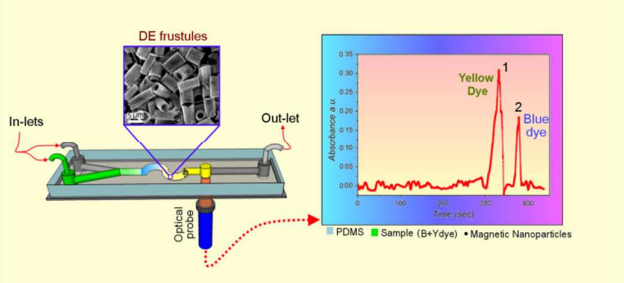
Graphical Abstract



A simple frit-free PDMS (polydimethylsiloxane) microfluidic chromatographic separation and detection device, packed with diatomaceous earth (DE) microparticles as a normal phase stationary material using iron oxide magnetic nanoparticles is described.

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Frit-free PDMS Microfluidic Device for Chromatographic Separation and On-Chip Detection

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A simple frit-free PDMS (polydimethylsiloxane) microfluidic chromatographic device, packed with diatomaceous earth (DE) microparticles as a normal phase stationary material using iron oxide magnetic nanoparticles is described. The separation of two model dyes was successfully demonstrated by integrating on-chip fibre optic detection to create a lab on a chip type of devices.

Microfluidics offers unprecedented advantages to design of small devices, with complex functions, improved accuracy, simple use, low energy consumption and high throughput.[1] These features, especially portability, low-cost and time effective analysis with ultra-low volume of analytes (µL to nL), make microfluidic devices highly attractive candidates in analytical chemistry. Liquid chromatography (LC) is recognized as one of the most powerful separation techniques in analytical chemistry due to its proven high separation efficiency, reliability and versatility. There is a strong trend in recent years to integrate LC into microfluidics in several ways to develop various chromatographic devices in micro scale including capillary liquid chromatography (CLC), capillary electro chromatography (CEC),[2] open-tubular columns,[3] polymer or silicon-based monolithic columns,[3, 4] microfabricated pillar columns,[5] and packed columns.[6] These new microchip based LC techniques are under continuous development, mainly focusing on improvement of their specific features such as a microchip design, the separation efficacy of stationary phase, flow systems, sensitivity of detection method and specific applications. However there is lack of integrated approaches to combine these improvements into one step to considerably improve the performance of LP microfluidic devices.

One of the main practical challenges in conventional and microchip based LC is to retain stationary phase of micropartices inside the microchip channels or microcolumns during packing and separation process. Several methods and approaches were developed to solve this problem by using the frit,[7] physical barriers,[6, 8] tapered capillary,[2, 9] valve systems[10] and bottle neck geometries.[11, 12] However, there are number of problems associated with these methods such as higher cost, complicated fabrication process, instability of stationary phase, air-bubble formation and band broadening during the operation.[2, 13] Hence, new technical solutions are urgently required to improve packing and retaining stationary phase and its stability during LC operation.

Synthetic silica microparticles are one of the most widely used stationary phase materials for packaging LC columns.[14] Silica particles have high mechanical strength, high thermal and chemical stability, high surface area and easily tailored particle size, pore size and surface chemistry for various separation applications.[14, 15] The silica packing material (C18) functionalized with organic silanes is still accepted as a benchmark in LC. However there are some disadvantages associated with synthetic silica particles such as high-cost, high energy and time consumption, the use of toxic materials and the toxic wastes associated with it. To address some of these limitations and improve the overall separation capability of LC columns several other nano and micro scale materials were explored in the past including synthetic alumina particles, porous carbon, carbon nanotubes (CNTs),[16] silica aerogel,[11] and sol-gels.[17]

Nature elegantly solves molecular separation and purification problems by synthesising nanostructured materials of complex 3-D geometries, through a simple and unique self-assembly process. Thus these natural materials could be ideal candidates for LC separations. Diatoms, microscopic unicellular algae are one of the most outstanding examples of Nature's ability to produce 3-D porous silica nanomaterials with ordered pores, organized in a specific hierarchical pattern.[18] Hence the naturally occurring silica shells (frustules) from fossilised single-cell algae called diatomaceous earth (DE) is proposed in this work to be used as a stationary phase as a much cheaper material, which could provide competitive separation performance compared with conventional synthetic silica. DE have distinct architecture of porous silica shells with highly ordered pore structures.[18-20] Diatoms with their 'pill-box' structure, a large inner hollow space, micro and nanoscale porosity, high surface area, amorphous silica, tailorable surface chemistry, low density and proven biocompatibility makes them an excellent candidate for LC stationary phase. Diatom nanotechnology has recently emerged, offering many striking applications in optic, photonic, sensing and biosensing,

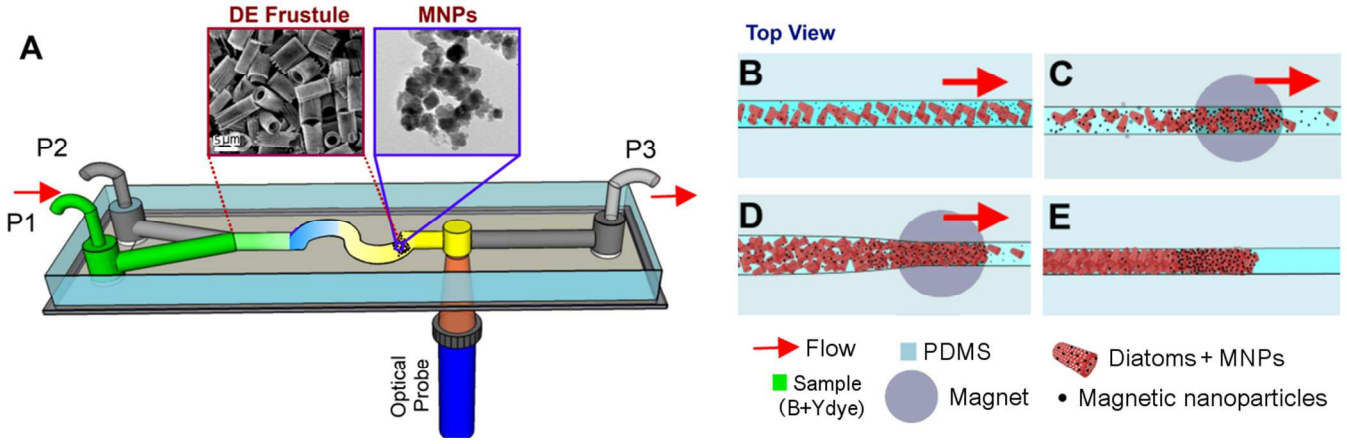

Fig. 1 (A) Schematic of developed PDMS microfluidic device which consists: P1 and P2 are for liquid/sample entries, P3 is for the exit, a stationary phase packed with frit-free DE particles (frustule) achieved by magnetic nanoparticles (MNP) stacked on the end of channel by magnetic field and connected fiber optic for direct detection of separated molecules; (B-E) scheme describes the preparation of frit-free packing of the DE silica microparticles: using MNPs; (B) The first step: flow of DE, MNPs and MNP embedded DE particles, (C) Second step: magnetically arresting the flowing particle, (D) Third step: the slight expansion of PDMS microfluidic channel due to hydrodynamic pressure and (E) Fourth step: the formation of stable packed channel with DE particles upon drying and ready for separation.

molecular separations, filtration, immunoisolation, micro and nanofabrication, catalysis and drug delivery.[18-24]

In this paper we have applied an integrated approach to improve the overall performance of LC based microfluidic device focused on several aspects including: a simple microchip design using PDMS, ease of packing the stationary phase, new type of stationary phase based on natural silica particles and integration of the chip with optical fiber optic detection system. Hence the aims are three-fold, the first is to introduce diatom silica particles combined with microfluidic channel made from PDMS as a stationary column. To the best of our knowledge the use of DE silica as a stationary phase in the microfluidic channel for LC separation has not been reported in the literature. The second aim is to demonstrate a novel method for packing the stationary phase, which involves of using magnetic nanoparticles (MNPs) and external magnet which provides stable and frit-free packaging of stationary phase. Third aim is to integrate microchip separation device with on-chip detection system using UV/Vis fibre optic placed in the pre designed detection area of the microfluidic channel. The scheme of this integrated microchip chromatographic device is presented in Fig. 1A. The PDMS device were fabricated by using soft photolithographic techniques,[25] which consists of three ports in total, two ports P1 and P2 are used for the sample and mobile phase entry, the remaining port P3 is used for exiting the liquids from the channel. Fibre-optic probe linked to micro spectrophotometer (Ocean Optics, USA) and to the computer system was used for real time detection of separated analytes.

PDMS microfluidic device with 150 µm channel width, and 100 µm depth with 'S' shape and chambers of 1 mm diameter for the tubing insertion and for detection were fabricated using a standard photolithographic technique.[25] Diatom silica microparticles (DE) were prepared by purification of raw DE materials supplied from Mount Sylvia Pty. Ltd. (Australia).[21, 26] MNPs were synthesized using synthetic procedure previously described[27] to have the particle sizes around 40 to 65 nm which are smaller than the pore size of diatoms (300-400 nm in diameter). Typical SEM and TEM images of diatom structures and MNPs including their size distribution are shown in Fig. 1S and 2S respectively, Supporting Information. To pack the channel the MNPs and DE particles were mixed (3:1 w/w ratio) in methanol, which allows the MNPs to enter inside the hallow structure of DE to form MNP embedded diatoms. The scheme of packing DE stationary phase inside microfluidic channel is presented in Fig. 1B-E. The mixture of these particles were initially flown at 50 µL/min flow rate inside the PDMS device, followed by placing an external magnet underneath the device just below the channel (Fig. 1C) at the position which is selected to be the end of packing. MNPs and MNP embedded diatom particles were magnetically arrested in the channel which forms a keystone physical barrier for incoming particles allowing only the liquid to flow through because of the high porous structure of the diatoms. The process of creating keystone effect by using MNPs is presented in Supplementary Information as Video 1 and 2. Once the physical barrier is created, the subsequent injection of known amount of pure diatom particles suspended in methanol at higher flow rates (200 µL/min) was packed the desired length of the channel with diatom particles.

Furthermore PDMS is a soft material having Young's modulus of 0.5 to 4 MPa,[6] which can deform relatively at low hydrodynamic pressure, while packing the channel, the pure diatoms were injected from methanol solvent at relatively high flow rate of 200 µL/min, because of the keystone effect, the pressure would build up inside the channel making the side walls of the PDMS to slightly expand as shown schematically in Fig. 1D. Once the flow of the liquid is stopped, the PDMS walls push the stationary phase inwards, making a very tight packing of the diatom material which is called *clamping effect,*[6] upon drying the device at 60 ^{o}C in the oven overnight, the stationary phase was stable and intact in the channel, the stationary phase was intact even at very high flow rate (tested up to 800 µL/min flow rate). This innovative approach facilitates the packing of the channel with stationary phase without of any frits, integrated physical barriers, tapers, restrictors and bottle neck type of designs and we believe it presents an important contribution to address this problem.

Cross section of the prepared PDMS chromatographic microchannel presented in Fig. 2 to provide evidence of tight sealing and the close packing of the DE particles at interface of PDMS wall. SEM images of the typical structures of the diatom silica microcapsules packed inside microfluidic channel are presented in Fig. 2A-B. The images show a characteristic structure of porous silica particles from diatom species called *Aulacoseira sp.* with perforated cylindrical shell closed or a half opened at one end with regularly spaced rows of pores of

diameter 300–400 nm. The average size of DE particles used for packing was confirmed using a dynamic laser light

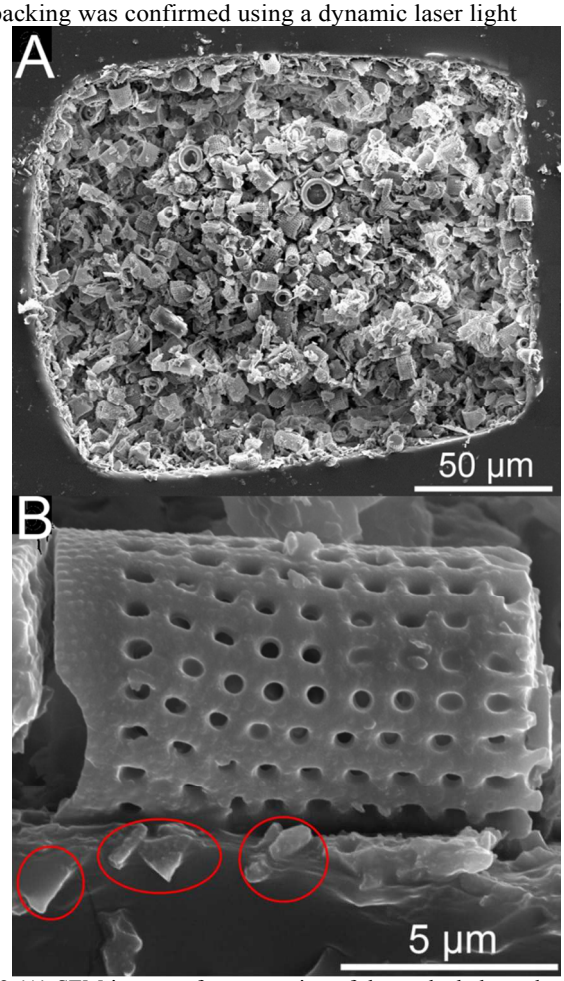

Fig. 2 (A) SEM images of cross section of the packed channel and (B) SEM image of typical structure of a single DE particle (frustule) in contact with the PDMS wall showing intact packing of the particles.

scattering (DLS) method showing a near bell shaped size distribution curve with the central peak around 10 µm (Fig. 1S, Supporting Information). The DE material has high specific surface area of 21.02 m^2/g and the total pore volume is 94.60 mm^3/g, which mainly comes from the pores on the diatom skeleton (Fig 2B). (The adsorption/desorption isotherm and BET plots are presented in Supporting Information as Fig. 3S and 4S respectively). Upon releasing the pressure during packing procedure, due to contraction of the PDMS channel walls, *anchoring effect* can be seen where the particles have partially pierced in to the wall (red circles) of the PDMS channel (Fig. 2B). It is well known that, in smaller LC channels, the *wall effect* is prominent depending on the shape, size and their interaction of the packing particles and the column, which often leads to the poor packing of the stationary phase and hence the poor separation efficiencies.[28, 29] Contrarily in our PDMS channels with DE particles as packing material we obtained tightly bonded particles to the PDMS surface, where the *wall effect* is completely minimised with improved *clamping* and *anchoring effects*. Because of simplicity and low-cost this approach presents important contribution to address existing problems with frit-based packaging of chromatography columns in microfluidic and conventional designs.

Separation capability and the efficiency of the chromatographic microfluidic device were demonstrated by using, two food dyes FD&C Blue No 1 (Brilliant blue, $C_{37}H_{34}N_2Na_2O_9S_3$) and FD&C Yellow No 5 (Tartrazine, $C_{16}H_9N_4Na_3O_9S_2$) supplied by (McCormick, USA).[6, 11, 13] These dyes were selected based on their slightly different polarity and molecular size. Their chemical structure are presented in Supporting Information as Fig. 5S. Dyes were premixed and injected into the device from the port P1 (Fig. 1) at a flow rate of 100 µL/min at room temperature. The photographic time profile separation of these dyes in the microfluidic channel is presented in Fig. 3A-D.

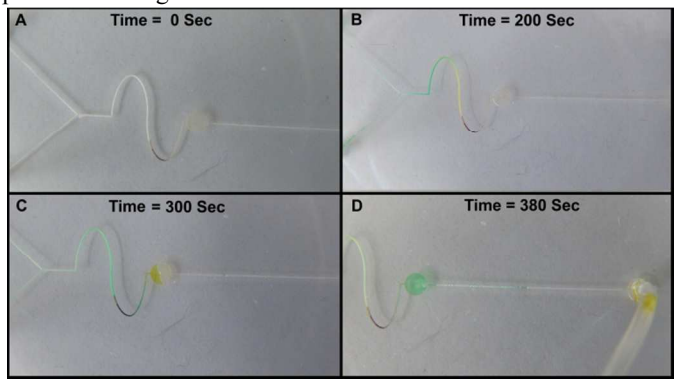

Fig. 3 Time scan light microscopic images taken during separation process: (A) 0 sec of dye injection, (B) at 200 sec the dyes have been separated in DE packed channel, (C) at 300 sec the first separated yellow dye has occupied the detection area and (D) both the dyes have separated from each other.

The syringe pump connected to the outlet P3 of the chip was run in reverse to suck the liquid at the flow rate of 700 µL/min while at the same time eluting the channel with open port P2 which was connected to milli-Q water reservoir. The pre mixed dyes started separating with each other in less than 200 sec in the channel, which is visible by naked ayes. The blue dye with a larger molecular size and polar interfacial properties, which make stronger interactions with the stationary phase was retained longer time in the column. The yellow dye being smaller and less polar was eluted from the channel faster before even crossing the 'S' part of channel. The DE particles are expected to have improved chromatographic characters compared with the conventional silica particles because of their porosity and unique 3-D structures and this experiment clearly demonstrated their applicability of the chip-based normal phase chromatographic separation.

The on-chip detection of the separated dyes was achieved by micro fibre optical probe operating in UV/Visible spectral range which is attached to the device (Fig. 1A). The absorbance spectra from the detection spot on the chip were acquired at two wavelengths, 420 nm and 630 nm simultaneously by using Spectra Suite software (Ocean Optics, USA). The typical chromatogram collected after complete separation of individual dyes from each other is presented in Fig. 4. The yellow and blue dyes were reached the detection area in 300 and 380 sec, respectively. Control experiments using the microfluidic device without of DE stationary phase for the same mixture of dyes resulted in only one sharp peak in UV/Visible spectrum (Fig. 4 inset). Further, the separation efficiency of the microfluidic for Blue and Yellow dyes was determined as 5.7×10^5 and 8.4×10^3 with the peak resolution between the individual peaks of 1.72. Continuous polymer beds with isopropyl groups yield efficiencies of 3.5×10^5 for uracil.[4] Gasper et al[11] have reported the similar dyes separation in 6 sec at flow rate of 12nL/s in 1 mm column. Over all, the separation efficiencies of our device are comparable and promising[2] for further applications.

In conclusion, a new micro-chromatographic device packed with natural porous silica from diatoms in a unique way without

frits and physical barrier by using novel packing method involving magnetic nanoparticles (MNPs) is demonstrated. It

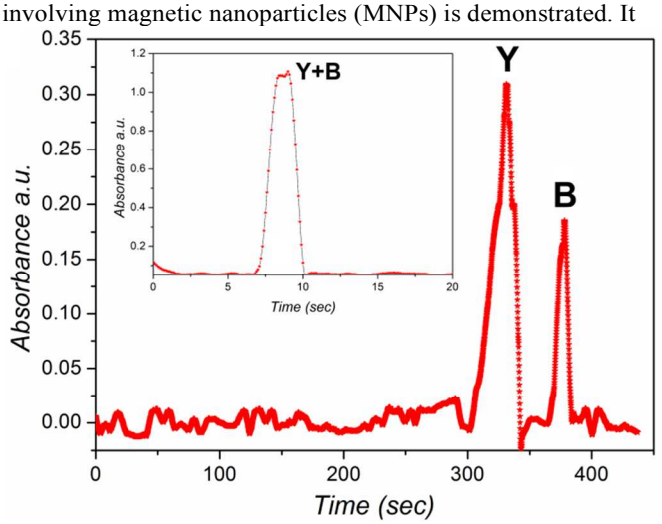

Fig. 4 Functional characterization of fabricated microchip separation device showing typical chromatogram of two separated model molecules (blue and yellow dyes) using on-chip detection by UV/Vis microfiber optical probe: Inset is the chromatogram of mixed dye when injected without the DE stationary phase in the microfluidic channel, showing no separation.

was shown that the diatom particles can successfully replace conventional synthetic silica particles and can be used as an inexpensive material for stationary phase and broad LC applications. PDMS and diatom microparticles showed to have strong interfacial interaction between each other which minimises the *wall effect* commonly seen in conventional LC columns. This device is integrated with on-chip detection assisted by UV/Visible micro fibre optical probe and channel design, makes the device lab-on-a-chip type of device. Finally the separation of two model molecules was successfully demonstrated with separation efficiency comparable with conventional LC columns. By tailoring surface chemistry with specific functional groups it is possible to tune separation selectivity of DE silica particles for separation towards specific organic molecules. This, very simple portable and inexpensive device has potential use in broad applications including in biomedical, pharmaceutical and environmental analysis and especially for development of demanding point-of-care diagnostic systems.

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Notes and references

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Electronic Supplementary Information (ESI) available: SEM, TEM, BET, particle sizes of DE and MNPs. See DOI: 10.1039/c000000x/

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