Centrifugally driven sample extraction, preconcentration and purification in micro-fluidic discs (μ-CDs)

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Abstract

Centrifugally driven micro-fluidic discs (μ-CDs) have attracted significant interest within the analytical science community over the last decade, primarily being focused on the potential of such platforms for performing parallel and/or multiplex biological assays and further application in biomedical diagnostics. More recently, μ-CD based devices have also been applied to environmental analysis as platforms for multi-sample extraction and transportation, prior to off-disc analysis in the laboratory. Therefore, this review critically summarises recent developments with μ-CD platforms for sample extraction, preconcentration, fractionation and purification in bioanalytical and environmental applications. In addition to also summarising the common methods employed in the fabrication of μ-CD platforms, as applications of μ-CDs in sample extraction are generally based on enclosed series of extraction phases/micro-columns, the preparation of these stationary phases in micro-fluidic channels embedded in μ-CDs is also discussed.

Keywords

Centrifugally driven micro-extraction, micro-fluidic discs (μ-CD), lab-on-a-disc, sample extraction, preconcentration, fractionation, purification, packed stationary phase, monolithic stationary phase, electrophoretic separations.

1. Introduction

New trends in the development of analytical instrumentation continue to focus on system miniaturisation and automation, in obvious efforts to meet current demands for in-situ or on-site analysis, and within cutting edge technology areas such as point-of-care diagnostics. Portability, long-term maintenance-free operation, low-power demands, integrated sample preparation and analysis, are the desired performance characteristics for this type of device. Micro-fluidic devices appear to offer a number of advantages in the race to achieve these targets. Indeed, micro-fluidic platforms have already become well established in many areas of modern science and technology, with their inherent capacity for improved mass and heat transport, speed of analysis, low sample/reagent volume requirements, and ability to integrate several analytical operations in a single device (e.g., extraction, separation, reaction, incubation, detection, etc). Furthermore, the small dimensions of micro-fluidic chips facilitates their complete integration into more complete miniaturised and portable instrumentation [1].

Compact disc (CD) based micro-fluidic platforms (μ-CD), also occasionally known as "lab-on-a-CD" or "lab-on-a-disc" (LOD), present several unique features which makes them attractive for integration within portable systems [1-4]. Most significantly, the use of centrifugal forces for fluid propulsion presents a clear advantage of μ-CD platforms over other micro-fluidic devices, as no connections to external pumps or power supplies are needed, which simplifies enormously the design of the whole system. Parallel sample processing and/or multiplexing analysis is also possible as many individual structures can be typically replicated radially within a single disc. Additionally, samples can be reproducibly split into multiple individual chambers by

designing micro-fluidic networks which allow the connection of metering chambers to a supply channel and a waste reservoir, as shown by Lutz *et al.* [5]. This represents a clear advantage in terms of reproducibility in sample handling, pre-treatment and analysis, as all samples in the disc are processed under the same conditions, as well as in terms of time and costs of the analysis. Furthermore, additional forces resulting from spinning the CD can be used to advantage. Such is the case of the Coriolis force, which can be used to control the direction of the flow at the junction of a channel with two other symmetrical channels [6] or chambers [7]. Spinning the disc alternatively in opposite directions, i.e. clockwise and anti-clockwise, has also been successfully used as a simple but effective method for improving the speed of mixing [8, 9].

All of the above features have contributed to the growth and expansion that centrifugally driven micro-fluidic devices has experienced in the last 15-20 years, within both the academic and industrial communities. Several commercial analytical systems based on μ-CD platforms are available in the market from companies such as Abaxis, Gyros AB, Quadraspec, and Samsung. A number of interesting reviews on the topic report the full application history of μ-CD based devices for sample preparation (e.g. sample lysis, plasma separation), nucleic acid amplification and analysis, immunoassays, DNA micro-array hybridisation, cell-based assays and optode-based ion sensors [1-4, 10, 11]. Among these applications, sample preparation deserves special consideration as it can be the source of significant error due to sample loss or cross-contamination between samples. A popular application of μ-CD devices in the area of sample preparation has been the separation of plasma from red blood cells in whole blood, which is an essential step in the automation of an immunoassay. Such isolation of target analytes from the sample matrix is often

necessary to avoid interferences from other components present in the sample. In many cases a preconcentration step is also required when dealing with analytes that are present at very low concentrations, commonly the case in environmental analysis. Those techniques are typically based on the use of a stationary extraction phase, such as packed or monolithic micro-columns, upon which the analyte is ideally selectively retained.

The content of this review covers highlights from recent applications of μ-CD to analyte extraction, preconcentration and purification, many of which use on-disc extraction phases of varying dimensions and formats. Additionally, work focused on the combination of centrifugal and electrokinetic pumping for sample fractionation will be also discussed. To begin the review, an overview of the most common fabrication techniques employed in the production of μ-CD platforms, together with the preparation of stationary phases within micro-channels is presented.

2. Fabricating micro-fluidic discs

A number of established and contemporary methods for the fabrication of micro-fluidic platforms, both for standard chips and μ-CDs, can be found within the literature. The techniques used for channel formation in these devices can be divided into seven main categories, namely (1) computer numerical control (CNC) milling; (2) xurography; (3) laser ablation; (4) soft lithography; (5) hot embossing; (6) injection moulding; and (7) thermoforming. Most of these techniques leave open channels which have to be closed by an additional layer. Closing may be performed either by thermal, adhesive, mechanical based bonding techniques or a combination thereof.

CNC milling is a rapid prototyping technique relatively inexpensive and, therefore, widely used in academic research. Several hard polymers have been used in the fabrication of μ-CDs by CNC milling, which demonstrates the utility of this technique, in terms of the substrate. For example, Puckett e*t al.* fabricated eight replicated micro-structures into 3-mm-thick poly(methyl methacrylate) (PMMA), later utilised for simultaneous protein-ligand binding assays [12]. These micro-structures consisted of several reservoirs connected by channels with different dimensions (635 μm wide and 635 μm deep; and 127 μm wide and 63.5 μm deep), all integrated in a μ-CD of 120 mm O.D. and centre hole of 15 mm diameter. Optically transparent adhesive tape was used to seal the system. Hoffmann *et al.* chose cyclic olefin copolymer (COC) as the disc substrate due to its optical properties, low permeability to moisture and temperature resistance [13]. A micro-channel network was milled directly into the 3-mm-thick disc, which integrated several reservoirs (16 mm x 5.1 mm x 5.1 mm) used for storage of buffer-filled glass ampoules. The ampoules were inserted manually into the milled storage reservoirs and then sealed by a slightly elastic self-adhesive film, typically used in polymerase chain reaction (PCR) applications.

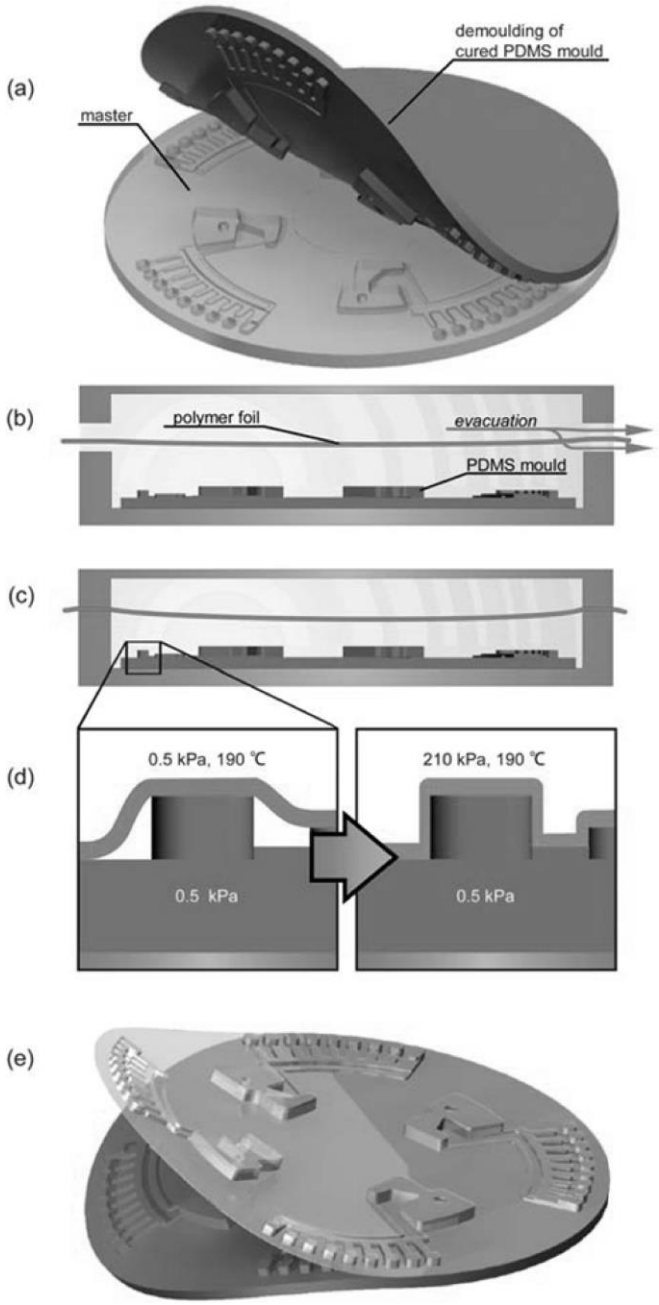

Figure 1. Steps followed in the fabrication of a μ-CD platform by thermoforming of COC films: **(a)** fabrication of the PDMS mould; **(b)** assembly in a modified hot embossing chamber and evacuation; **(c)** vacuum; **(d)** application of a pressure difference at moulding temperature; **(e)** demoulding after cooling, venting and opening the process chamber. Reproduced with permission from ref. [24]. Copyright 2010 The Royal Society of Chemistry.

The combination of CNC milling and xurography has also become popular for the fabrication of μ-CDs. Lafleur *et al.* have recently utilised this combination for fabrication of a polymeric μ-CD-based system for in-field environmental sampling [14]. Conventional CNC milling was used to produce reservoirs in blank uncoated 0.6-mm-thick conventional CDs and 1.2-mm-thick DVDs, both based on polycarbonate (PC), or alternatively in 6.35-mm-thick PMMA. Low aspect features such as channels and vents were created by xurography in 100-μm-thick adhesive films with a cutting plotter. To close the structures, PMMA or quartz discs were used as top layers, the latter being required to ensure maximum transmission in the UV region. After careful alignment, the three different layers were bonded with a hand crank cold laminator.

A cutting plotter was also used in a recent study to pattern a glycol-modified polyethylene teraphthalate (PETG) layer, which was then laminated between two separate PETG layers at 130 °C, each having a thickness of 125 μm [15]. The micro-fluidic architecture, which was designed for PCR applications, was used to passively compartmentalise a DNA sample into 1000 wells of 33 nL by centrifugation. It should be noted that xurography is a technique really suited for fast prototyping, however, unlike in CNC milling, suitable substrates for this technique are limited to thin films and, thus, the height of the micro-structures is typically determined by the thickness of the films employed. Thus, stacking of several layers is sometimes required to achieve larger channel dimensions.

A micro-fluidic platform developed for the recirculation of large volume samples in 4 mm x 80 μm channels (cross-section) was actually produced by a multi-layer

technique using different layers of PMMA, double-sided pressure sensitive adhesive (PSA) and COC [16]. Micro-fluidic channels were ablated in the PSA films (thickness, 80 μm) using a CO_2 laser. A thermal laminator at 60 °C was then used to bond the layers after proper alignment.

One of the most common micro-fabrication techniques reported for production of μ-CDs in academic research is still soft lithography [17-21]. This replication technique, mostly used in the production of polydimethylsiloxane (PDMS) chips, is relatively inexpensive and, more importantly, allows the precise fabrication of features even in the order of a few microns. Silicon-based masters (moulds) produced by standard photo-lithography are typically employed for casting PDMS. This method has been employed in the fabrication of CD-like PDMS micro-fluidic chips for many different applications such as protein crystallisation [17], DNA hybridisation [18], plasma extraction from whole blood [19], and rapid discrimination of single-nucleotide mismatch (SNM) [20]. Alternatively, Penrose *et al.* employed a PMMA master, which had been produced by micro-machining, for casting PDMS [21].

Hot embossing [22] and injection moulding [9, 23] are also replication techniques that have been used for μ-CD fabrication, although rather costly and time consuming, if only small number of discs are required. However, plastic injection moulding can be useful in small-scale production of discs when good dimensional repeatability or a high degree of complexity is required. A good example of a highly complex platform was introduced by Lee *et al.*, for fully-automated testing of infectious diseases from whole blood samples [9]. This platform consisted of PMMA injection moulded top and bottom plates, bonded together using a UV adhesive.

Very recently, thermoforming of films has been introduced as a method for μ-CD fabrication [5, 24]. A complex μ-CD designed for the development of a genotyping assay by real-time PCR is a good example of this process (Fig. 1) [24]. Here a master was first micro-milled from 4.0-mm-thick PMMA. A special grade of PDMS pre-polymer (Elastosil RT-607) was then cast onto this master and cured at 80 °C for 30 minutes. After demoulding, the PDMS mould with a bulk thickness of 3.5 mm was cured at 200 °C and 1 kPa for one hour. This PDMS mould was in turn used as the master onto which a COC film (thickness, 188 μm) was thermoformed using a hot embossing system to produce the final micro-fluidic structure. For the thermoforming process itself, the COC film is heated well above its glass transition temperature and simultaneously pressed against the PDMS for 15 minutes, as shown in Fig. 1. After cooling and demoulding, the micro-fluidic network transferred to the COC film was then sealed with PSA to produce the final μ-CD.

3. Preparation of extraction phases in μ-CDs

3.1. Packed stationary phases

Packed columns have been applied in micro-fluidic devices for chromatographic and electro-chromatographic separations, as well as for sample extraction and enrichment. In centrifugally driven μ-CD based devices both reversed-phase columns and affinity columns of diverse nature and functionality, have been employed for matrix removal and selective retention of target species [14, 25-29].

Traditional extraction columns, comprised of well defined cylindrical channels, are typically packed utilising external high-pressure pumps. Micro-fluidic devices are often unable to withstand such pressures, due to the materials and bonding techniques used in their fabrication. In addition, the cross-section geometry of micro-fluidic channels is generally non-perfectly semi-circular, or rectangular, instead presenting asymmetrical corners, again resulting from the fabrication procedure employed. Tallarek *et al.* demonstrated that these deviations from regular geometries affect the packing structure and, consequently, the porosity distributions and the fluid flow velocity profiles [30]. Therefore, a lower column-to-column reproducibility is generally expected in columns packed in micro-fluidic channels compared to capillary packed columns.

Centrifugal forces have been utilised for consolidation of packed beds within micro-fluidic channels embedded in μ-CDs. A strategy for packing C_8 reversed-phase micro-columns (particle size, 5.8 μm) by centrifugal forces in PDMS CD chips was presented by Penrose *et al.* [21]. The width of the micro-channel to be packed (cross-section, 1 mm x 250 μm) was gradually reduced at the outlet end to approximately 100 μm in order to retain the particles *via* a "keystone" effect. The packing material, introduced in the channel as an ACN slurry was allowed to accumulate under gravity prior to consolidation and equilibration of the column by spinning the disc at 2700 rpm. A stable packing structure was obtained with consolidated bed lengths ranging from 20 to 22 mm into the 32-mm-long channel.

Other groups have also reported protocols to pack micrometer-size beads in μ–CD channels based on slurry-packing by centrifugal forces [14, 25, 31], and alternative

means to retain the beads during packing, such as the use of quartz wool column frits [14, 25]. Gong *et al.* also showed that centrifugal forces can be effectively used to packed sub-micrometer silica beads within micro-fluidic channels, although here within a standard micro-fluidic chip rather than within a μ-CD [32]. Beads with an average diameter of 320 nm were successfully packed from 2 % slurries (w/v) at 9000 rpm, resulting in stable columns with high packing density and minimal defects.

3.2. Monolithic stationary phases

Despite the increasing number of publications in the last decade demonstrating the utilisation of monolithic materials as stationary phases, micro-reactors and micro-mixers within diverse micro-fluidic platforms [33] , as yet the integration of monoliths in centrifugally driven μ-CDs has been rather unexplored. To date, only one work has recently demonstrated the *in-situ* preparation of a monolithic column in the micro-channels of a PDMS/PC disc for protein purification [34]. In this work, the channel network was fabricated in PDMS, and then reversibly bonded to a PC cover. The monolithic column was fabricated in a chamber of 2.5 μL, which was connected to the sample and the waste reservoirs through a 150-μm-wide and 200-μm-deep main channel (Fig. 2). A side micro-channel connected to the lower part of the column chamber was used to introduce the polymerisation solution (3 μL) ensuring the complete filling of the column chamber prior to polymerisation. The methacrylate monolith was then prepared *in situ* by microwave-initiated polymerisation of a mixture containing 2-hydroxyethyl methacrylate (HEMA), [2-(methacryloyloxy)ethyl]trimethylammonium chloride (META), ethylene dimethacrylate (EDMA), 2,2′-azobis(isobutyronitrile) (AIBN) and cyclohexanol/dodecanol. To allow covalent attachment of the monolithic column to

the channel walls, formation of silanol groups in the column chamber upon UV irradiation of the PDMS surface was followed by silanisation with 3-(methacryloyloxy)propyl]trimethoxysilane (MPTS). The resulting highly porous monolithic column was then washed with the testing buffer to remove any unreacted monomers, prior to any analytical extractions being performed with the μ-CD based device. To the best of the authors' knowledge, this work is rather unique in the use of microwave-initiated polymerisation for the fabrication of monoliths within micro-fluidic channels.

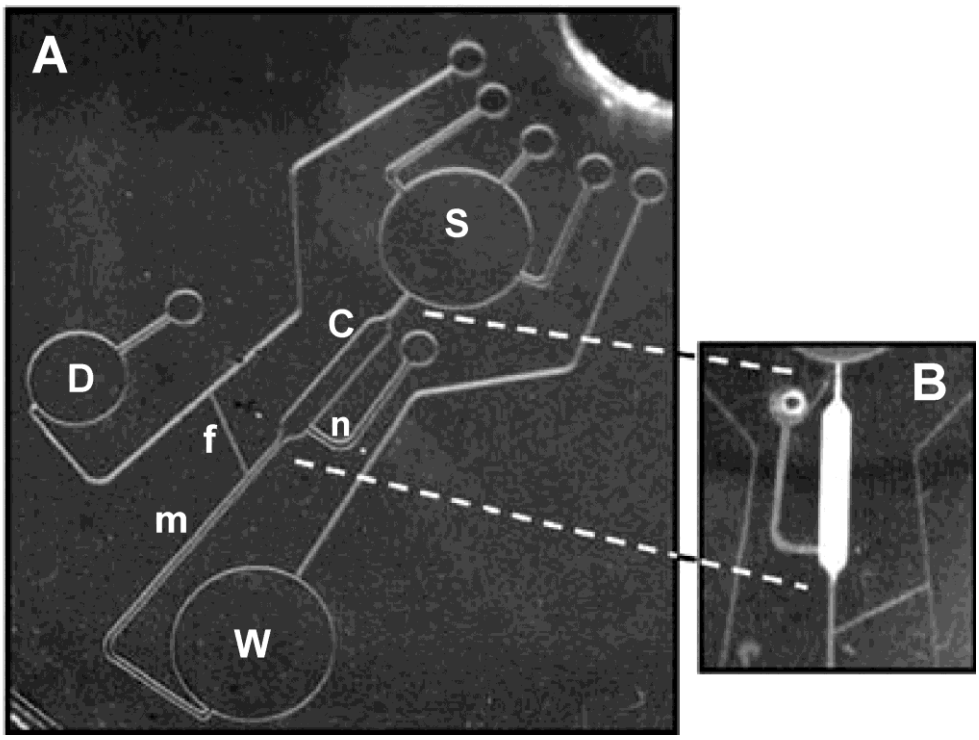

Figure 2. (A) Photograph of the micro-fluidic network fabricated in PDMS including sample chamber (S), column chamber (C), waste chamber (W), main micro-channel (m), side micro-channel connected to column chamber for loading polymerisation solution (n), fractionation chamber (f), isolation and detection chamber (D). **(B)** Photograph of the monolith prepared *in situ* in the column chamber. Reproduced with permission from ref. [34]. Copyright 2006 Springer-Verlag.

4. Electrophoretic separations for sample fractionation in μ-CDs

Electrophoretic techniques have been transferred to micro-fluidic formats for the isolation and separation of a wide range of analytes. A significant contribution to the success of this approach lies in one of the techniques key features, i.e. the use of the electroosmotic flow (EOF) for electrokinetic injection and flow control, which avoids the need for external sample injectors or pumps, thus, facilitating system integration and miniaturisation. Spěšný and Foret showed that the combination of electrophoretic separations with centrifugal pumping in a μ-CD is also possible [23]. In this work, the micro-fluidic network was first patterned on a PC disc by injection moulding, and a thermal-bonding process was then used to seal the channels with a polyolefin film. A hydrophilic zigzag-shaped channel (cross-section, 100 μm x 50 μm) was used for injection and subsequent separations by isotacophoresis (Fig. 3). Gel plugs prepared from a mixture of 1 % agarose with the leading and terminating electrolytes were placed at the inlet and outlet of the separation channel, respectively. Similar plugs were prepared with the sample mixture, which was electrokinetically injected. Once the analytes were electrophoretically separated, the disc was spun at 1500 rpm for 5 s to burst hydrophobic valves to narrower side channels, located at the junction of each of the zigzag-shaped channel turns, the former connecting the separation channel with individual reservoirs. The collected fractions could then be stored in the sealed individual reservoirs, or alternatively passed through a C_{18} packed column located at the bottom of each reservoir, until further analysis.

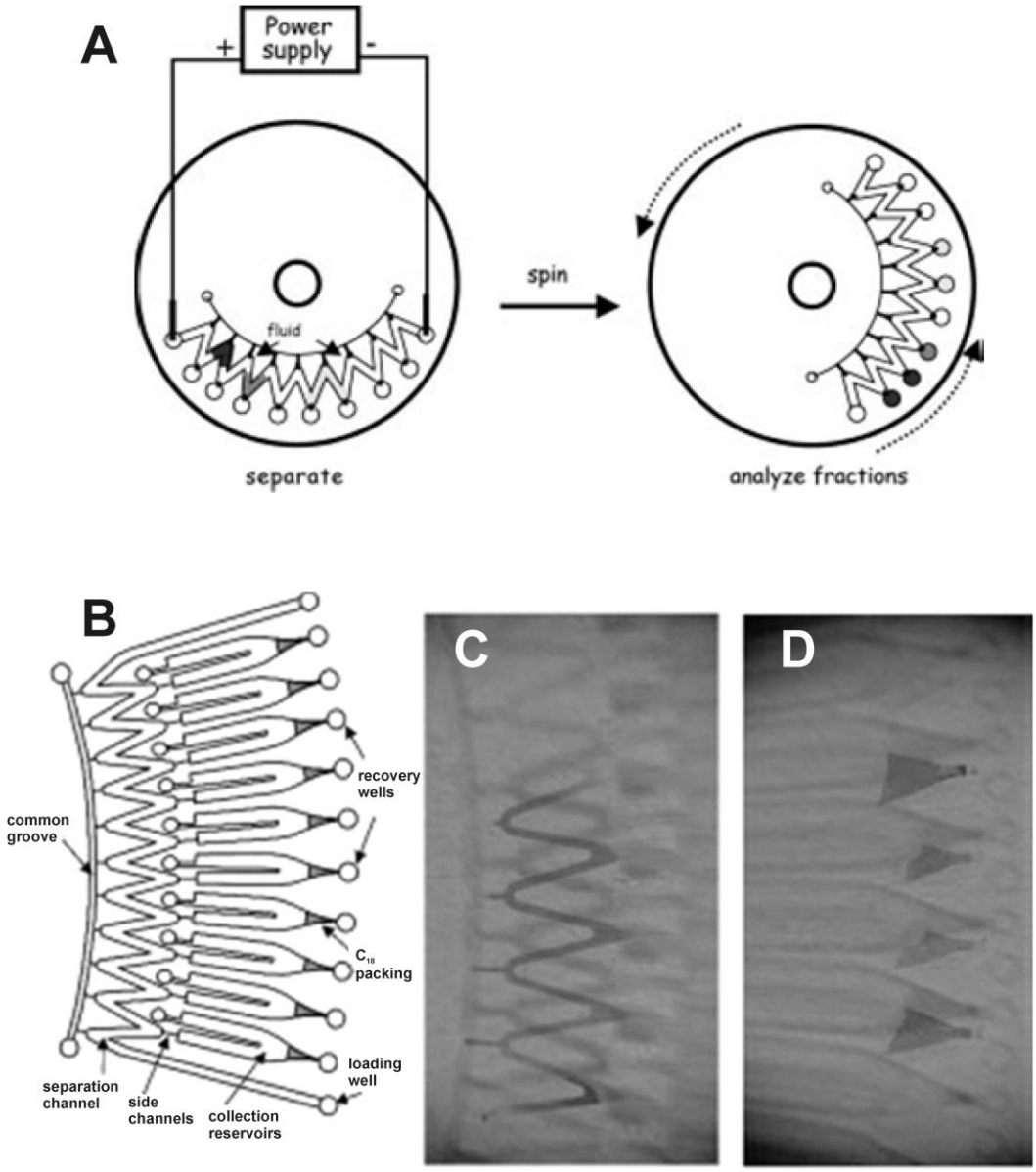

Figure 3. (A) Scheme showing the operation of the μ-CD platform consisting of application of potential for electrophoretic separation followed by CD spinning for fraction collection. **(B)** Scheme showing the structure of the micro-fluidic network. Snapshots taken during electrophoretic separation **(C)** and after fraction collection **(D)**. Reproduced with permission from ref. [23]. Copyright 2003 Wiley-VCH Verlag GmbH & Co. KGaA.

A second interesting design produced within a μ-CD, combining centrifugal and electrophoretic forces, has been reported by Wang *et al.* [35]. In this case a concentric conducting circuit, wired to the power supply was fabricated on a PC plate by metal sputtering using a customised mask. The micro-fluidic network was then patterned on the PC substrate by photo-lithography, producing a layer of photo-resist of 35 μm. The channel structures were then closed with a top PC plate integrating the access reservoirs by thermal bonding. Results obtained for some basic separations indicated that the separation efficiency was effectively increased when the electrophoretic and centrifugal forces had opposite directions. Therefore, more efficient separations could be performed by increasing the separation time in shorter channels.

5. Applications

5.1. Environmental analysis

Environmental analysis represents one of the largest application areas for all formats of solid phase extraction (SPE). In elemental analysis SPE is commonly used for preconcentration and matrix removal, often utilising high capacity ion exchange resins or speciality chelating resins in the case of trace metal determination. A recent example of this approach on a μ-CD platform has been reported by Lafleur and Salin [25]. The group has developed a novel μ-CD device for field sampling and on-site preconcentration of V, Pb, Ni, Cu and Co in drinking water samples. Metal-8-hydroxyquinoline complexes, formed off-disc were adsorbed onto multiple C_{18}-bonded silica gel packed micro-columns (approximately 10 mm in length and 1 mm width) housed within the μ-CD, utilising centrifugal forces to load the samples. The micro-fluidic extraction platform was evaluated using 300 μL volume samples, a significant reduction in sample volume to that generally required with conventional

SPE procedures. No elution procedure was developed as direct laser ablation (LA) of the extraction phase was used to directly vaporise the analytes from the μ-CD column, for introduction into an inductively coupled plasma mass spectrometer (ICP-MS). Multiple sample extractions could be performed simultaneously, as each disposable disc could accommodate a total of 8 SPE units. The paper reported detection limits of between 0.3 and 2 ppb for V, Pb, Ni and Cu using this approach.

The same group have also utilised similar μ-CD designs for the solid phase extraction and preconcentration of both fluorescein and anthracene in aqueous samples [14], and the solid – liquid extraction of pyrene from soils [36]. In the former case the extraction column was packed with C_{18} silica gel and on-column simultaneous front-face fluorimetry and absorbance measurements were obtained. Extracted fluorescein was visible as a bright yellow slug concentrated on the SPE micro-column, with an absolute detection limit of 20 ng for a 140 μL sample quoted for anthracene. For on-disc solid – liquid extraction the group developed a novel magnetically actuated extraction unit coupled with an in-line filter unit and on-disc detection. Fig. 4 shows the schematic design and expanded images of the μ-CD developed. The extraction of soil samples was achieved using hexane and results of on-disc extraction compared with standard solvent extraction within a conventional separation funnel. Following extraction, an on-disc detection limit of 1 ppm pyrene, and an extraction yield of between 78-84%, were quoted.

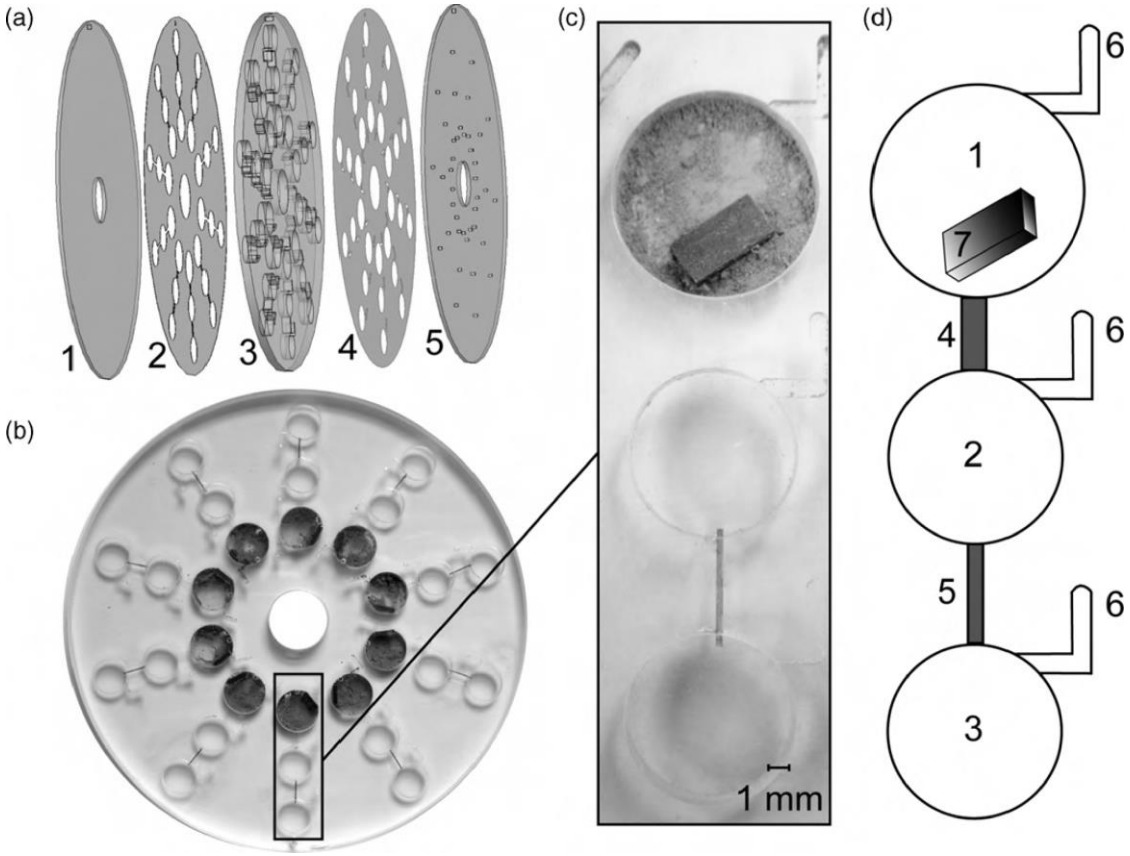

Figure 4 (a) Scheme showing assembly of the micro-fluidic disc: 1) PMMA bottom layer, 2) adhesive layer with chambers and rectangular passive valves, 3) PMMA layer with chambers and cylindrical passive capillary valves, 4) adhesive layer with chambers, 5) top PMMA layer with vents; **(b)** picture of the final μ-CD platform containing 10 micro-structures; **(c)** enlarged view of a single micro-structure; **(d)** scheme showing the operation units integrated in each micro-structure: 1) magnetically actuated liquid–solid extraction unit, 2) filtration unit, 3) detection unit, 4) rectangular passive valve, 5) cylindrical passive capillary valve, 6) vent holes and vent lines, 7) magnet. Reproduced with permission from ref. [36]. Copyright 2010 Elsevier.

Although not involving an extraction or preconcentration phase, Salin and co-workers have also recently developed a μ-CD design for the simultaneous determination of

nitrite and nitrate, which utilises on-disc chemical reduction and derivatisation, and also offers direct on-disc detection [37].

5.2. Bioanalytical applications

5.2.1. Protein purification

As mentioned in Section 3.2, Moschou *et al.* used an ion-exchange methacrylate-based monolithic column integrated in a PDMS/PC μ-CD for protein isolation and purification (Fig. 2) [34]. Various solutions of enhanced green fluorescent protein β-lactamase (EGFP) were used as samples. Once the sample (4 μL) was introduced into the sample chamber, the disc was spun at 850 rpm to drive the solution into the column chamber for protein retention *via* ion-exchange interactions with the monolithic stationary phase. The monolith was then washed with 7 μL of buffer to remove any non-specifically bound species. Bound protein was eluted with 5 μL of eluting buffer forced to the isolation chamber through a narrow side micro-channel (fractionation channel) by spinning the μ-CD at 1,150 rpm. Recovery values were then calculated by measuring fluorescence intensity using a fiber optic fluorometer (λ_{ex} 488 nm, λ_{em} 508 nm). Recovery values ranging from 85 % to 93 % (n = 10) were obtained for three different columns/μ-CDs once the monolith composition was optimised. The whole process (binding, elution and detection) was complete in ca. 3 min.

5.2.2. Immunoassays

Immunoassays are currently one of the main application areas of μ-CDs. The well known advantages offered by the micro-fluidic assay (e.g. shorter analysis times, shorter incubation times, smaller sample/reagents volumes), make this approach very

attractive. Additionally, μ-CDs offer the possibility of integrating tens of assays in one single device.

In a relatively recent publication, Honda *et al.,* demonstrate the high-throughput capabilities of the immunoassay-based Gyrolab Bioaffy™ μ-CD platform, produced by Gyros AB [29]. The 12-cm-diameter μ-CD contains 104 identical micro-columns packed with streptavidin-functionalised polystyrene beads. The 15-nL-volume packed columns retain biotinylated antibodies that are then used for the capture of the analytes (α-fetoprotein, interleukin-6 and carcinoembryonic antigen) present in 200-nL-volume samples. The captured antigens are then detected following its interaction with fluorophore-labelled secondary antibodies by laser-induced fluorescence. In this case, complete analysis of the 104 samples, including sample loading, incubation and washing steps, detection and signal processing is complete in only 50 min.

The high throughput assessment of antibody specificity and affinity was also demonstrated using the same platform. In this application, His_6-tag proteins were captured on three different commercial metal ion affinity chromatography (IMAC) resins (particle size range, 16-34 μm) previously packed in the μ-CD micro-channels [28]. The analysis of the target antibodies was then completed using a similar approach as above, *via* a fluorescence sandwich immunoassay.

5.2.2.1. Medical diagnostics

Immunoassays are standard tools in clinical diagnostics and thus, significant efforts are focused on the implementation of such assays in portable, simple-to-operate, micro-fluidic-based devices for application in decentralised point-of-care (POC)

diagnostics. A few examples of μ-CDs applied to medical diagnostics can be found in the literature. For instance, Nagai *et al.*, presented a μ-CD designed for the analysis of secretory immunoglobulin A (sIgA), which is a biomarker for mental stress present in saliva (Fig. 5) [38]. Although not demonstrated with real samples, the authors showed that the μ-CD chip was capable of measuring sIgA in 1-μl-volume standard samples at the low concentrations of interest *via* a competitive antigen-antibody reaction with fluorescence detection. The PDMS μ-CD integrated 108 individual micro-channels containing a reaction chamber (diameter, 1.5 mm) in which a single 1-mm-diameter glass bead functionalised off-chip with the antigen was embedded. The novelty of this work was the use of a single-bead as the stationary phase for the antigen-antibody reaction in an effort to reproducibly control the amount of antigen previously immobilised on the bead surface, and thus, improve the reproducibility of the assay.

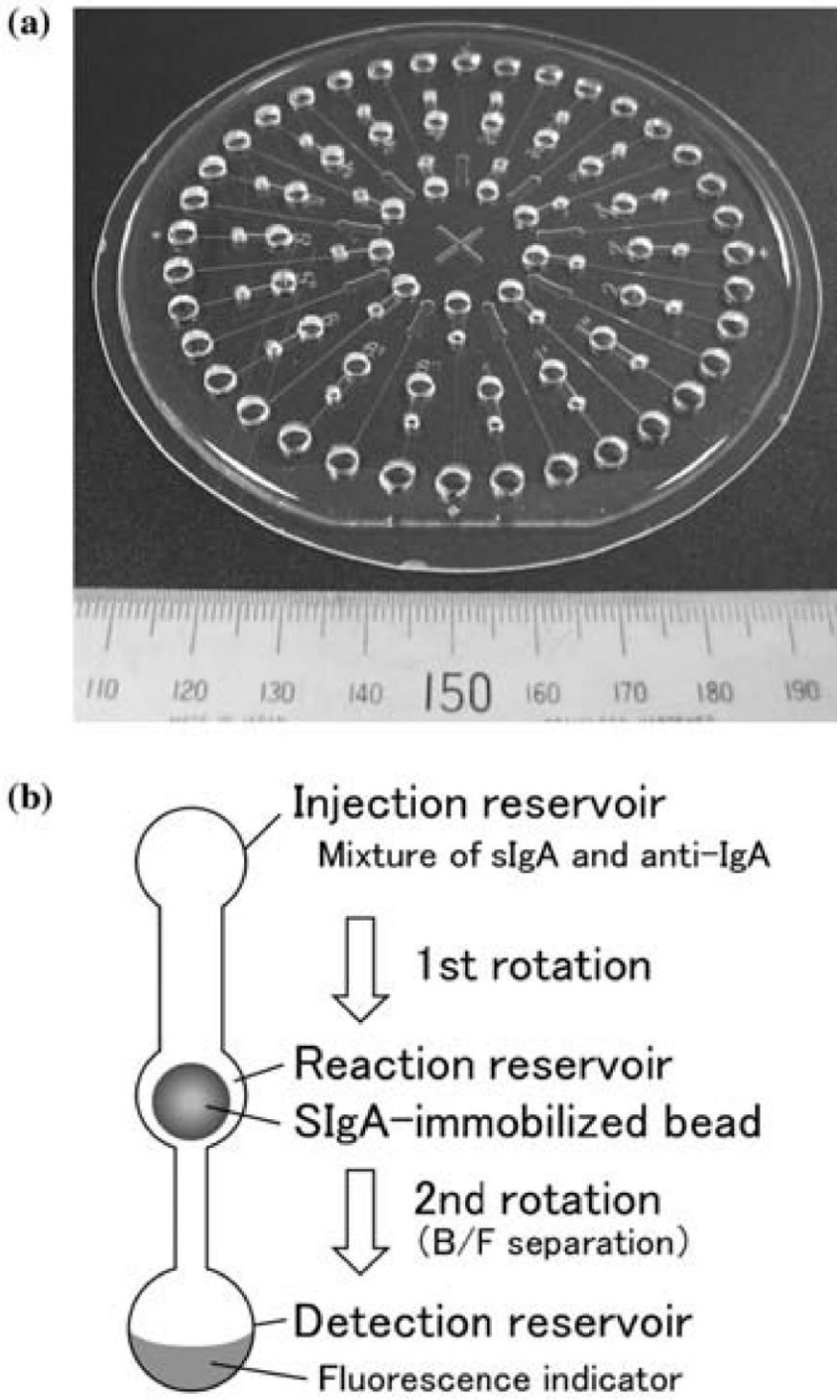

Figure 5. (a) Photograph of the micro-fluidic network fabricated in PDMS**; (b) s**cheme showing the pattern of a single micro-structure and the methodology employed for detection of sIgA. Reproduced with permission from ref. [38]. Copyright 2007 The Japan Society for Analytical Chemistry.

A more complex, fully automated platform for detection of the Hepatitis B virus (HBV) in whole blood, based on a bead-suspension immunoassay, was recently reported on by Lee *et al.* (Samsung) [9]. This system consisted of a portable blood analyser and a PMMA μ-CD chip integrating 3 identical micro-structures capable of simultaneously measuring the concentrations of the virus antigen (HBsAg) and antibody (Anti-HBs), and a control sample (Fig. 6). Once the disc was loaded into the portable blood analyser, the automated analysis included separation of the plasma from the whole blood, and transfer of the plasma to the reaction chamber where incubation with target specific antigen- or antibody-functionalised polystyrene beads took place. The bead suspension was confined in the reaction chamber by a weir structure. The total analysis time, including several washing and reaction steps, together with absorbance detection was less than 50 min for the 3 simultaneous assays. A complex system of laser irradiated 'ferrowax' micro-valves (LIFM) based on iron oxide nano-particles dispersed in paraffin wax (response time, < 1 s) were used to control the delivery of the samples to the different chambers. A key feature of this device was the use of beads in suspension, which allowed the reduction of the incubation time due to the convective mixing of beads, sample and reagents resulting from repetitively spinning the disc in clockwise and counter-clockwise direction.

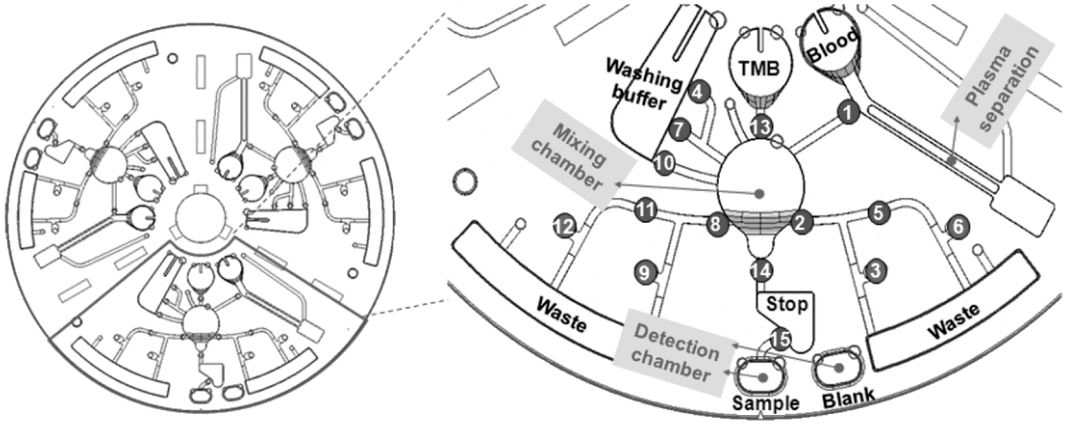

Figure 6. Left: scheme showing the design pattern of the micro-fluidic network fabricated on PMMA discs for detection of HBV. Right: enlarged view of a single micro-structure with numbers indicating the order in which the LIFM were operated. Reproduced with permission from ref. [9]. Copyright 2009 The Royal Society of Chemistry.

5.2.2.2. Multiplex Detection

A recent publication by Riegger *el al.*, demonstrated how μ-CD based platforms integrating columns packed with colour-labelled polystyrene beads could be used for multiplexed detection in fluorescence immunoassays [39]. Beads were again functionalised off-disc with various capture proteins. The bead suspension (mean size, 150 μm) was then packed into the detection chamber, which had a depth only slightly bigger than the bead size in order to enforce a monolayer distribution of the beads, therefore ensuring individual optical access to all the beads. The location and colour of each bead was determined using image-processing software and a colour CCD camera. Fluorescence intensity of each bead resulting from the reaction of the fluorescently-labelled detection antibodies with the captured target antigen was measured. The authors estimated that the number of immunoassays that could be

simultaneously detected by this system was about 15 when using dyes for the colour-encoding of beads, and about 5 when using luminescent quantum dots as colour tags.

5.2.3. Sample preparation for on-chip MALDI-MS

Gyros AB (Sweden) has also commercialised several μ-CD systems (i.e. the Gyrolab MALDI SP1 CD and the Gyrolab MALDI IMAC1 CD) for sample preparation prior to matrix-assisted laser desorption/ionisation mass spectrometry (MALDI-MS). Both of the above systems enable the simultaneous processing of 96 samples, but differ in the type of column used for target preconcentration and desalting, followed by elution and crystallisation within MALDI target areas (200 μm x 400 μm). The Gyrolab MALDI SP1 CD utilises reversed-phase micro-columns for retention of peptides from protein samples digested off-chip [27], while the Gyrolab MALDI IMAC1 CD uses IMAC micro-columns for retention of phosphorylated peptides from phosphoprotein digests [26].

5.2.4. DNA extraction

Recently, a ready-to-use μ-CD for DNA extraction was presented by Hoffman *et al.* [13]. The disc, fabricated in COC as described in Section 2, integrated all the components included in a commercial DNA-extraction kit, i.e. three glass ampoules filled with washing and elution buffers, and a silica extraction matrix (Fig. 7). For DNA extraction, 32 μL of pre-lysed blood was loaded in the channel inlet, followed by rotation of the disc, flushing the sample through the extraction matrix, and ampoule disruption for washing and elution steps. On-disc DNA extraction yield after 140 days of buffer pre-storage was still 77 % of an off-disc reference extraction. Additionally, the above method facilitated reagent storage at temperatures below the

freezing point. No liquid loss or damage to the glass ampoules was observed after storage at -20 °C for 60 days.

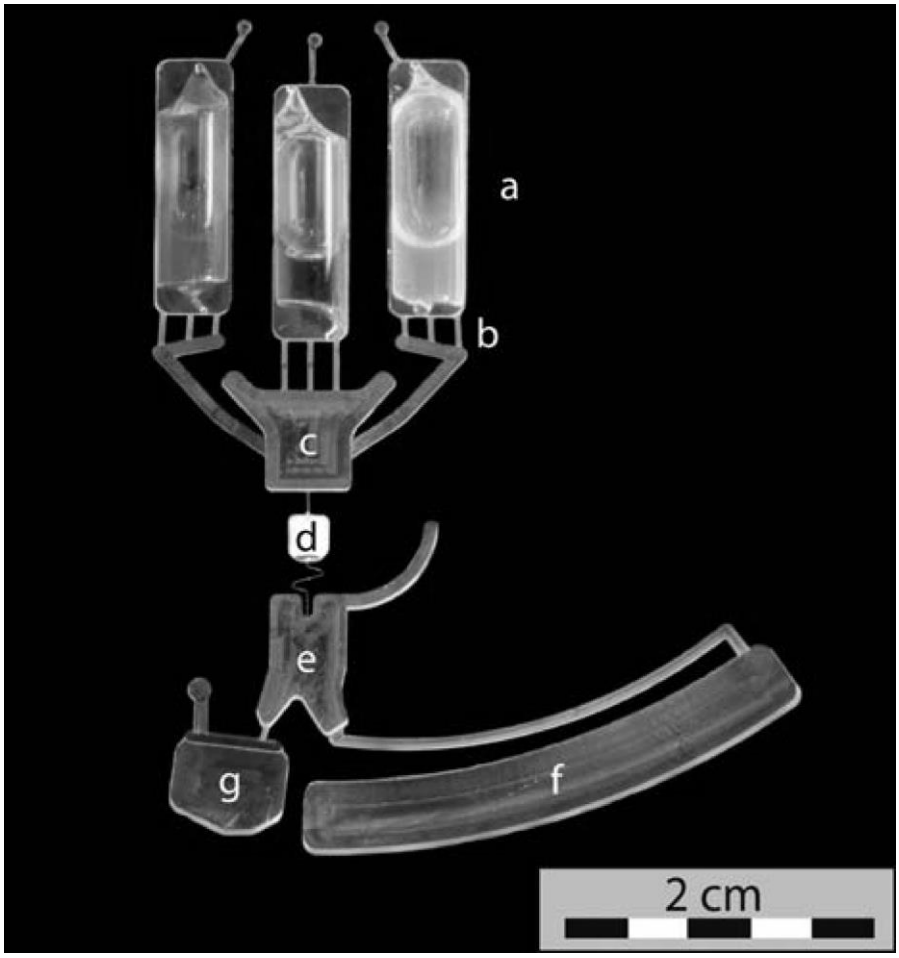

Figure 7. μ-CD platform for on-chip DNA extraction integrating (a) three glass ampoules containing washing and eluting buffers, (b) filter structures for retaining glass shivers, (c) sample chamber, (d) silica extraction matrix, (e) liquid switching chamber, (f) waste chamber, and (g) isolation chamber for purified DNA. Reproduced with permission from ref. [13]. Copyright 2010 The Royal Society of Chemistry.

Concluding remarks

This review presents the latest developments and achievements using μ-CD based approaches for sample extraction, preconcentration, purification and fraction collection. It is clear from the literature reviewed herein, that even though many applications are still in the area of bioanalysis, centrifugally driven μ-CD platforms are finding their way into other application areas, due to the inherent advantages this technology presents. In particular, both miniaturisation and high-throughput, parallel sample processing is particularly suited for on-site environmental analysis.

Some promising emerging technologies include the use of monolithic columns as stationary phases in μ-CDs, although still relatively few publications have appeared, in contrast to their considerable utility in alternative micro-fluidic platforms. Likewise, impressive μ-CDs combining electrophoretic and centrifugal pumping for separation, preconcentration and quantitative fraction collection were first reported several years ago, however little further development has been realised in this area since.

In the area of μ-CD fabrication, the utilisation of relatively novel micro-fabrication technologies, i.e. thermoforming, opens new possibilities for realisation of ever more complex structures. Specifically, thermoforming technology appears to be a promising approach for production of low-cost, ready-to-use and disposable centrifugal discs containing pre-stored liquid and dry reagents. No doubt these recent advances will contribute to expand the possibilities and applications of CD based micro-fluidic technology in the near future.

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