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# Microfluidic chips with reversed-phase monoliths for solid phase extraction and on-chip labeling

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ABSTRACT

The integration of sample preparation methods into microfluidic devices provides automation necessary for achieving complete micro total analysis systems. We have developed a technique that combines on-chip sample enrichment with fluorescence labeling and purification. Polymer monoliths made from butyl methacrylate were fabricated in cyclic olefin copolymer microdevices and used for solid phase extraction. We studied the retention of fluorophores, amino acids and proteins on these columns. The retained samples were subsequently labeled with both Alexa Fluor 488 and Chromeo P503, and unreacted dye was rinsed off the column before sample elution. Additional purification was obtained from the differential retention of proteins and fluorescent labels. A linear relation between the eluted peak areas and concentrations of on-chip labeled heat shock protein 90 samples demonstrated the utility of this method for on-chip quantitation. Our fast and simple method of simultaneously concentrating and labeling samples on-chip is compatible with miniaturization and desirable for automated analysis.



## 1. Introduction

The integration of multiple functions in a single device can result in faster, cheaper and improved analysis compared to traditional laboratory methods [1]. Many such processes have been integrated in microfluidic devices, including extraction/purification [2–6], labeling [7,8], preconcentration [9,10], microdialysis [11,12], and detection [13,14]. One of the greatest difficulties in achieving completely miniaturized and integrated analysis has been the step of sample preparation [15], although important progress is being made in selected areas as noted below. Importantly, solid phase extraction (SPE) has been used in integrated sample processing, including extraction, purification and preconcentration [2,5,6].

SPE is a common sample preparation method wherein analytes are retained on a solid support and are subsequently eluted in a concentrated form [16]. The most common SPE modes in microfluidics are affinity [4,17,18] and reversed-phase [6,19,20]. Affinity SPE in microchips has been used to extract and quantify four cancer biomarkers in blood [4], to preconcentrate and purify PCR products [17], and to extract thiazole orange-conjugated adenosine monophosphate [18]. Reversed-phase columns are useful in the extraction of non-polar to moderately polar compounds. Silica-based materials are common reversed-phase SPE supports, having been used for the extraction of parabens and fluorescent dyes [6], the preconcentration of peptides and cytochrome c [19], and the concentration and separation of Rhodamine 123 and fluorescein isothiocyanate (FITC)-labeled ephedrine [20]. Monolithic columns are seeing increased usage because they can be easily prepared on-chip without the need for retaining structures like frits [5,21], and the porosity and surface area can be tuned by varying the monomer/porogen composition [22]. Neutral methacrylates are generally hydrophobic enough for reversed-phase SPE [23,24]. Cyclic olefin copolymer (COC) is a preferred polymer material for SPE microchips because of its stability in organic solvents such as acetonitrile that are used for elution [24,25]. Though photografting is generally used to modify the microchannel for enhanced monolith/wall adhesion [24], it has also been shown that monoliths fabricated in COC devices can be stable without surface pretreatment [26].

The integration of SPE with capillary electrophoresis or microchip electrophoresis (μCE) offers the advantages of improved sensitivity and sample cleanup, along with shorter analysis times, reduced sample loss and automation [20,27]. Typically, when SPE is coupled to μCE, an interface is used to control the transfer of analytes from the SPE column to the separation channel. In the analysis of dopamine by SPE-μCE, polydimethylsiloxane (PDMS) microvalves were used to segregate the processes of extraction, rinsing, sample elution and separation [23]. In a different setup, a nanoporous membrane sandwiched between two PDMS layers was used as an electrokinetic valve to separate the processes of SPE and electrophoretic separation [20]. In these PDMS SPE-μCE systems, separation of small molecules was done, which is less complicated

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since they typically do not bind as much as proteins to the device walls [28].

Many samples do not fluoresce naturally and have to be derivatized to take advantage of the superior sensitivity of laser-induced fluorescence detection. Labeling is often performed off-chip, but on-chip labeling has been achieved both in pre-column [7,8,29] and post-column [30–32] formats. Conventional dyes with high quantum yield, such as Alexa Fluor and fluorescein, are often used to label analytes. Additionally, fluorogenic reagents [7,8,33], which are weakly fluorescent until they react with a primary amine, have been used for on-chip derivatization because they produce lower background fluorescence, their reaction kinetics are fast, and they do not change the electrical charge of the sample [34]. The fluorogenic reagents, CE dye 503 [7], ThioGlo-1 [8], and naphthalene-2,3-dicarboxaldehyde [35], have been used for on-chip derivatization. While acceptable results were obtained from these integrated systems, lower limits of detection, avoiding on-line mixing of high concentrations of fluorescent dyes, and addressing system peaks and background fluorescence are all areas where improvement is desirable.

In this work we demonstrate a novel approach combining SPE with on-chip labeling and purification to improve over previous methods. We show that samples retained on a solid support can be concentrated and labeled on-chip prior to elution. Reversed-phase butyl methacrylate (BMA) porous polymer monoliths were formed in COC microdevices and used to study the retention of fluorophores, amino acids and proteins. The retained and concentrated samples were then labeled on-chip with Alexa Fluor 488 TFP ester or Chromeo P503. Subsequent rinsing to remove unreacted dye and selective elution of labeled sample relative to unconjugated fluorophore helped to greatly reduce the background fluorescence typically observed in on-chip labeling. On-chip labeling of heat shock protein 90 (HSP90) resulted in a concentration-dependent area of the eluted peak, demonstrating the ability of this method to quantify on-chip labeled samples. This work thus offers improved capabilities in on-chip labeling for miniaturized analysis.

## 2. Experimental

### 2.1. Reagents and materials

Zeonor 1020R (COC) was purchased from Zeon Chemicals (Louisville, KY, USA). Methyl methacrylate (MMA), BMA, lauryl methacrylate (LMA), 2,2-dimethoxy-2-phenylacetophenone (DMPA), 1-dodecanol, cyclohexanol, Tween 20, ethylene dimethacrylate (EDMA), and isopropyl alcohol were obtained from Sigma–Aldrich (St. Louis, MO, USA). Bovine serum albumin (BSA) was purchased from New England Biolabs (Ipswich, MA, USA) and HSP90 was from Sigma–Aldrich. The amino acids glycine, aspartic acid, phenylalanine and arginine were obtained from Sigma–Aldrich. The amino acids were labeled with FITC, while the proteins were labeled with Alexa Fluor 488 TFP ester. Both fluorophores were from Invitrogen (Carlsbad, CA, USA). Chromeo P503 was obtained from Active Motif (Carlsbad, CA, USA). Fluorescein (sodium salt) and dimethyl sulfoxide (DMSO) were purchased from Sigma–Aldrich. Hydroxypropyl cellulose (HPC, 100 kDa average molecular weight) was from Aldrich (Milwaukee, WI, USA). Sodium dodecyl sulfate (SDS) was obtained from Fisher Scientific (Pittsburgh, PA, USA). Buffer solutions were made from anhydrous sodium carbonate, sodium bicarbonate, acetonitrile (ACN), and sodium azide, all from EMD Chemicals (Gibbstown, NJ, USA). Amicon Ultra-0.5 centrifugal filter devices were obtained from Millipore (Billerica, MA, USA). All solutions were prepared with deionized water (18.3 MΩ cm) purified by a Barnstead EASYpure UV/UF system (Dubuque, IA, USA).

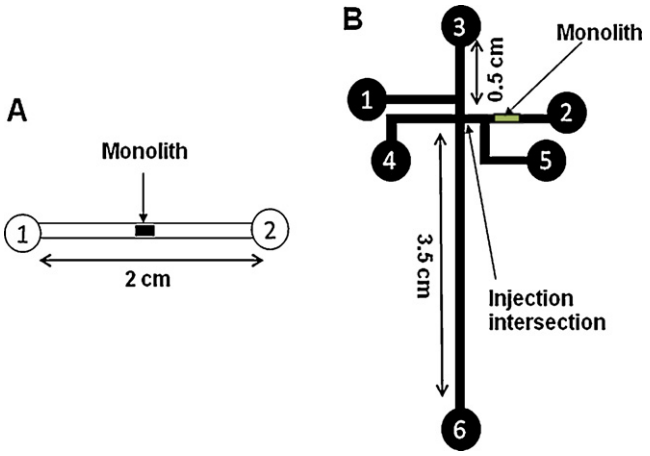


**Fig. 1.** Schematic of microfluidic devices used for on-chip labeling. (A) Simple, two-reservoir design used for initial testing. (B) Layout used for integrated experiments. The reservoirs are: 1 – sample, 3 – fluorescent dye, 4 – rinse buffer, 5 – eluent, and 2 and 6 – buffer. The lengths from reservoirs 1, 2, 4 and 5 to the injection intersection are all 0.5 cm.

### 2.2. Device fabrication

COC plates were obtained by cutting the sheets into 2 in. × 1 in. pieces with a bandsaw. Holes in the cover plate were then drilled to serve as reservoirs in the bonded devices. The microdevices were fabricated using a combination of photolithographic patterning, etching, hot embossing and thermal bonding as described previously [36]. Bonding of COC was done at 110 °C for 20 min. Two different microchip designs were used for these experiments. A simple, two-reservoir layout (Fig. 1A) was used for initial testing while the design in Fig. 1B was used for integrated experiments where no exchange of liquids in reservoirs was required. The channels in both designs were ~15 μm deep and ~50 μm wide. Before polymerization of a monolith, the channels were rinsed with isopropyl alcohol.

### 2.3. Preparation of SPE monoliths

Monoliths were made from a solution consisting of 25% BMA (or MMA or LMA), 15% EDMA, 25% (w/w) dodecanol, 10% cyclohexanol, and 25% Tween 20. 1% DMPA was added to the mixture as photoinitiator. The solution was sonicated for 10 min and degassed for 5 min. It was then filled into the device, and a mask was used to expose only the desired portion of the chip to UV radiation. Exposure was carried out with the use of a SunRay 600 UV floodlight from Uvitron International (West Springfield, MA, USA) at 50 mW/cm$^2$ for 10 min. A 2 mm long monolith was formed in each microdevice in the location indicated in Fig. 1. After polymerization, devices were rinsed with isopropyl alcohol followed by buffer (10 mM carbonate, pH 9.3). The morphology of the monoliths was characterized using a Philips XL30 FEG environmental scanning electron microscope (SEM) from FEI (Hillsboro, OR, USA).

To determine the loading capacity of the monoliths, increasing concentrations of BSA were loaded on column using a syringe pump (Harvard Apparatus, Holliston, MA, USA) operating at 20 μL/min. First, the column was preconditioned with carbonate buffer containing 30% ACN, and then each BSA solution was loaded for 10 min (200 μL total volume), followed by a 5 min rinse with aqueous carbonate buffer. BSA retention was monitored via the background-subtracted fluorescent intensity at the CCD detector.