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Microchip-based electrochromatography: designs and applications

Martin Pumera *

Department of Chemistry, Universitat Autonoma de Barcelona, E-08193 Bellaterra, Barcelona, Spain

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Abstract

Different techniques and methods of electrochromatography on "lab on a chip" devices are reviewed. Described approaches include open-channel microchip electrochromatography relying on C_8, C_{18} and novel gold nanoparticle (GNP) coating of microchannel wall; packed-channel microchip electrochromatography with new ways of automated loading and unloading of conventional octadecylsilica beads; monolith-based microchip electrochromatography with tailored casting of stationary phase at the specific places of microfluidic network and novel photolitographically fabricated collocated monolithic structures. Specific issues related to the microchip electrochromatography, i.e. importance of high aspect ratio of the microchannels in the open-channel electrochromatography or approaches eliminating the wall effect in the monolith-based electrochromatography, are discussed. Various applications for environmental, pharmacological, genomic and proteomic analysis are described. The operation parameters of reviewed microsystems are summarized in easy-to-read tables.

Keywords: Electrochromatography; Microchip; Miniaturization; Coating; Packing material; Monolith; DNA; Peptides; Review

1. Introduction

The development of micro total analytical systems, also called "lab on a chip", has witnessed explosive growth in the recent years [1–8]. Chip-based devices offer attractive features, such as the potential of fabrication of highly multiplexed systems with zero-dead volume interconnections, automation, mass-production; all this together with high throughput analysis, low solvent/reagent consumption and low cost. Particular attention has been given to the development of microchip zone electrophoresis (μCZE) [9–11]. Although microchip zone electrophoresis offers high separation efficiency (with theoretical plate height ∼1 μm [12]), due to short separation channels (in order of millimeters/centimeters), there is a major need for additional separation mechanism that would manipulate selectivity in the chip-based separation systems. Such selectivity manipulation can be achieved by microchip electrochromatography (μCEC).

Microchip-based electrochromatography is a hybrid method of microchip zone electrophoresis and chip-based liquid chromatography (μLC) and it combines the best characteristics of both methods. Separation mechanism of μCZE is based on the difference between mobilities of solutes, while in μLC it is based on the differences of partition coefficients between two phases. Combining μCZE with μLC creates a powerful analytical tool capable to separate both ionic and neutral compounds. Furthermore, plug-like electroosmotic

Abbreviations: 2D, two-dimensional; AA, acrylic acid; AIBN, azo-isobutyronitrile; AMPS, 2-acrylamido-2-methylpropanesulfonic acid; *β*, aspect ratio; BODIPY, 4,4-difluoro-1,3,5,7,8-pentamethyl-4-bora-3a,4a-diaza-(S)-indacene; COMOSS, collocate monolith support structures; μCEC, microchip-based electrochromatography; m-μCEC, monolithic microchip-based electrochromatography; o-μCEC, open-channel microchip-based electrochromatography; p-μCEC, packed-channel microchip-based electrochromatography; μCZE, microchip-based zone electrophoresis; EOF, electroosmotic flow; GNP, gold nanoparticle; *H*, theoretical plate height; μLC, microchip-based liquid chromatography; MES, 2-(*N*-morpholino)-ethanesulfonic acid; MPTMS, 3-methacryloxypropyltrimethoxysilane; MS, mass spectrometry; ODS, octadecylsilica; PAH, polycyclic aromatic hydrocarbon; PDADMAC, poly(diallyldimethylammonium chloride); PDMS, poly(dimethylsiloxane); PSG, photopolymerized sol–gel; PMMA, poly(methyl methacrylate); RP, reverse phase; SDS, sodium dodecyl sulfate; SSA, 4-styrenesulfonic acid; StMA, stearyl methacrylate; Tris, tris(hydroxymethyl)methylamine; VSA, vinylsulfonic acid

* Tel.: +34 93581 2118; fax: +34 93581 2379.
E-mail address: pumera@email.cz.

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flow (EOF) profile results in reduced dispersion of the analyte zone, which increases column efficiency. Additionally, flow generated by electroosmosis obviates the use of high-pressure pumps, which are difficult to fabricate in the microchip manifold [13].

Since 1994, when the first μCEC work was published by Ramsey and co-workers [14], the microchip electrochromatography has received considerable attention. The aim of this review is to cover the time span of the last 11 years (1994–2004) while focusing on the main modes of μCEC; (i) open-channel electrochromatography (o-μCEC) in which the walls of microchannel are coated with stationary phase; (ii) packed-channel electrochromatography (p-μCEC) in which microchannels are filled with typical RP-HPLC packing materials; (iii) continuous rod electrochromatography in which channels contain monolithic stationary phase prepared by in situ polymerization or monoliths directly microfabricated using lithographic methods (m-μCEC).

2. Microchip electrochromatography: designs and applications

2.1. Open-channel electrochromatography (o-μCEC)

In o-μCEC the walls of a microchip channel are coated with the selector. Different distribution equilibrias between the running buffer and coated stationary phase are responsible for tailoring the selectivity and improved resolution of the solutes. As the diameter of the open channel decreases, the resistance to mass transfer decreases too [15] but other problems arise. For example, the smaller the surface of the modified channel is the more problems with the separation channel overloading are. Also injection and detection volumes must be scaled down proportionally to prevent excessive contributions to band broadening and therefore may fall bellow the limit of detection. To minimize these problems, channels with high aspect ratio (β) of the channel width to the channel height are fabricated (for an example, see Fig. 1). This allows improved mass transfer in one dimension while the sample load and detection volume is maintained in the other dimension [14]. The channel dimensions and the aspect ratios of reviewed o-μCEC articles are summarized in Table 1.

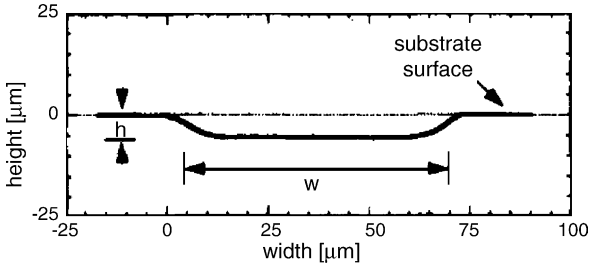

Fig. 1. Typical profile of channel cross section in open-channel microchip electrochromatography. High aspect ratio ($\beta = w_{1/2}/h$; where $w_{1/2}$ is the channel width at half-height, h is the channel height) improves mass transfer in one dimension while the detection is carried out in the other dimension. Reprinted from ref. [14] with permission.

2.1.1. C_{18} and C_8 reverse phase

The octadecyl reverse-phase coating is popular in microchip-based o-μCEC. The chemistry of the octadecyl (C_{18}) group bonding on a glass surface is well documented [16]. The first instance of microchip o-μCEC was described by Ramsey and co-workers [14]. They prepared glass microchip with serpentine column geometry and chemically-bonded octadecyl group (using chlorodimethyloctadecylsilane as a precursor) on the microchannel walls. To maintain high efficiency without sacrificing the detection path length or injection/detection volumes, the rectangular channel geometry with high aspect ratio $\beta = 12$ was used. Three neutral coumarin dyes were baseline separated in 170 s using effective separation channel length of 58 mm. For coumarin 440, plate height (H) 5 μm was observed, while the most retained component, coumarin 460, had $H = 45$ μm.

Another method for development of RP o-μCEC based on glass chip substrate was developed by Constantin et al. via sol–gel technique [17]. They produced stationary phase which contained silanol groups generating EOF and C_8 groups mediating the separation using tetraethoxysilane (TEOS) and octyltriethoxysilane as co-monomers. Separation of three polycyclic aromatic hydrocarbons was performed in 6.5 min. They compared the performance of chip-based o-μCEC with conventional capillary o-CEC coated by the same technique. Performance of the microchip μCEC was found to be slightly better in comparison to the conventional o-CEC [17].

Besides the glass-based devices with high aspect ratio channels, which are usually fabricated by chemical wet etching [14], the polymer-based substrates also offer possibility for manufacturing high-aspect ratio microstructures [18]. However, due to different technology of microfabrication of polymer chips the high aspect ratio is usually obtained by designing narrow and deep channels [19,20], contrary to glass-based isotropic etching technology, which allows to produce shallow and wide microchannels [14]. Polymeric poly(methyl methacrylate) (PMMA) microchip device for C_{18} reverse phase o-μCEC separation of DNA fragments was prepared by Soper and co-workers [19–22]. To modify the PMMA channel with C_{18} moiety, the bare PMMA surface was aminated with 1,2-diaminoethane or 1,3-diaminopropane. The following reaction with *n*-octadecane-1-isocyanate resulted to well-organized and highly crystalline C_{18} surface. While the unmodified PMMA is generally dissoluble by mobile phase containing acetonitrile, now the uniform C_{18} layer protected the underlying PMMA well. The C_{18}-modified PMMA was stable even upon long-term exposure to acetonitrile-based mobile phase. The EOF in C_{18}-modified channel was anodic because of unreacted amine groups. These resulted in an excess surface charge that was positive, producing the EOF reversed—compared to native