

an indication of the separated component, e.g. UV absorbance detectors where UV absorption is the signal, fluorescence detectors where laser induced fluorescence (LIF) is the signal, and the like.

The subject microchannels are suitable for use in the electrophoretic separation of the components of a variety of samples, including complex mixtures of proteins, carbohydrates and nucleic acids. Of particular interest is the use of the subject microchannels in the electrophoretic separation of complex mixtures of large numbers of differently sized nucleic acids, such as DNA fragments generated in large genome sequencing applications. Of particular interest is the separation of DNA fragments ranging in size from about 10 to 10,000,000 bp, usually from about 10 to 10,000 bp, more usually from about 10 to 5,000 bp. DNA detection and sequencing methods employing microchannels such as capillaries are extensively described in Barron & Blach, supra, § 6.6 and Lipshutz & Fodor, *Curr. Opin. in Struct. Biol.* (1994) 4: 376–380. The subject microchannels are particularly suited for such applications as they give rise to substantially reduced EOF and sample component adsorption as compared to native or untreated fused silica. By substantially reduced EOF is meant that the EOF occurring in the subject microchannels under electrophoretic conditions is generally less than about 50%, usually less than about 40%, more usually less than about 30% of the EOF present in native or untreated fused silica microchannels under similar conditions. In the subject microchannels, generally less than 20%, usually less than 15%, more usually less than 10% of the overall electrophoretic mobility of the charged entities moving through the channel under an applied voltage gradient will be attributable to EOF.

In addition to the subject microchannels and methods of their use in electrophoresis, kits are provided comprising the subject microchannels with a an electrophoretic medium, e.g. separation matrix, where the medium may be preloaded in the channel or separate from the channel. The kits may further comprise various detectable labels capable of providing a signal, usually fluorescence, which may be combined with a sample and provide for a detectable signal during or after electrophoresis, such as labeled nucleic acids, e.g. fluorescently labeled oligonucleotide primers, fluorescently labeled ddNTPs, and the like.

The following example is offered by way of illustration and not by way of limitation.

EXPERIMENTAL

Example 1

CE of dsDNA in Polymethylmethacrylate Capillaries

A 34 cm polymethylmethacrylate (PMMA) capillary having an inner diameter of 75 μm and an outer diameter of 375 μm was pressure-loaded with a separation media prepared from 0.4 g of hydroxyethylcellulose (HEC) (MW 90,000–105,000) and 1.5 g of hydroxypropylcellulose (HPC) (MW 300,000) dissolved in 98.1 g of 0.5 X TBE at 400 psi. A 10 base pair DNA ladder consisting of about 30 10-bp repeats (GibcoBRL) was loaded electrokinetically for five seconds at 5 kV. Electrophoresis was performed at 5 kV using a prototype electrophoresis instrument with a confocal fluorescence detector having Spindler & Hoyer (Medford, Mass.) optical components and an Omnicrome Argon Ion Laser operating at about 12 mW and 488 nm.

The results are provided in FIG. 1. Trace A is the separation of the 10 base pair ladder in a polymethacrylate

capillary, while trace B is the separation of an identical sample in a coated fused silica capillary of similar dimensions. Trace “A” has a positive baseline offset for comparison purposes only. Injection of a neutral marker was not detected within one hour of injection, indicating a negligible electroosmotic flow in both the polymethacrylate and coated fused silica capillaries. The tallest peak in each separation corresponds to the 100 base-pair fragment. Since any electroosmotic mobility would oppose the migration of the sample species toward the detector during the run, little electroosmosis was indicated by both the similarity and short run times of the two runs. Separations of an analogous sample in a bare fused silica capillary under similar conditions were of significantly poor quality and exhibited a reverse elution order.

It is evident from the above results and discussion that the acrylic microchannels according to the subject invention provide a viable alternative to microchannels prepared from fused silica in electrophoretic applications. The unmodified acrylic microchannels according to the subject invention give rise to EOF of sufficiently small magnitude so as to provide for good resolution of electrophoretically separated components, similar to the separation and resolution achieved with coated fused silica channels. As the subject microchannels require no surface modification, they are easier to prepare and use than surface modified channels. Furthermore, the optical transparency of the acrylic polymer material of the subject channels makes them amenable for use with on-line detection techniques. The subject microchannels are particularly suited for the separation of complex mixtures of large numbers of differently sized nucleic acids, such as labeled DNA fragments generated in genome sequencing applications.

All publications and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

The invention now being fully described, it will be apparent to one of ordinary skill in the art that many changes and modifications can be made thereto without departing from the spirit or scope of the appended claims.

What is claimed is:

1. In a method where charged entities are moved through a channel under the influence of an applied voltage differential, the improvement comprising the step of employing a microchannel formed from a surface of a polymethylmethacrylate material for reducing at least one of electroosmotic flow or adsorption as compared to a microchannel formed from native fused silica.

2. The method according to claim 1, wherein said microchannel is a groove in a substrate and said substrate is covered by a cover.

3. The method according to claim 1, further comprising the step of disposing an electrophoretic medium in said microchannel.

4. In a method where charged entities are moved through a channel under the influence of an applied voltage differential, the improvement comprising the step of employing a microchannel formed from a surface of an acrylic copolymer material for reducing at least one of electroosmotic flow or adsorption as compared to a microchannel formed from native fused silica.

5. The method according to claim 4, wherein said microchannel is a groove in a substrate.

6. The method according to claim 5, wherein said substrate is covered by a cover.