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Microchip electrophoresis with background electrolyte containing polyacrylic acid and high content organic solvent in cyclic olefin copolymer microchips for easily adsorbed dyes☆

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ABSTRACT

Plastic microchips can significantly reduce the fabrication cost but the adsorption of some analytes limits their application. In this work, background electrolyte containing ionic polymer and high content of organic solvent was adopted to eliminate the analyte adsorption and achieve highly efficient separation in microchip electrophoresis. Two dyes, rhodamine 6G (Rh6G) and rhodamine B (RhB) were used as the model analytes. By using methanol as the organic solvent and polyacrylic acid (PAA) as a multifunctional additive, successful separation of the two dyes within 75 μm id. microchannels was realized. The role of PAA is multiple, including viscosity regulator, selectivity modifier and active additive for counteracting analyte adsorption on the microchannel surface. The number of theoretical plate of 7.0×10^5/m was attained within an effective separation distance of 2 cm using background electrolyte consisting 80% methanol, 0.36% PAA and 30 mmol/L phosphate at pH 5.0. Under optimized conditions, relative standard deviations of Rh6G and RhB detection (n = 5) were no more than 1.5% for migration time and 2.0% for peak area, respectively. The limit of detection (S/N = 3) was 0.1 nmol/L for Rh6G. The proposed technique was applied in the determination of both Rh6G and RhB in chilli powder and lipstick samples with satisfactory recoveries of 81.3–103.7%.

1. Introduction

Microchip electrophoresis (MCE) has been proved to be an efficient separation technique for fast analysis. Its advantages such as reduced sample and reagent consumption, and the convenience of miniaturization make it an ideal candidate for field-deployable on-site analysis [1–3]. Glass and silica are the widely used substrates of microchips due to their excellent chemical compatibility and broad acceptance in chemical laboratories but the fabrication of glass or silica microchips is complicated and costly. To simplify the fabrication and reduce the cost of microchips, plastics have been widely used as substrates of microchips due to their great availability and well established fabrication methods for various shapes and structures [4]. By proper selection of the plastic, some special requirements such as biocompatibility and surface charge can be directly satisfied with bare plastic materials without any surface modification [5,6]. However, most of plastic materials with excellent optical and mechanical properties suffer from drawbacks such as non-specific adsorption of hydrophobic molecules and biomacromolecules, which frequently results in non-uniform and unstable electroosmotic flow (EOF) as well as serious loss of sensitivity and separation efficiency. Therefore, surface modification is frequently indispensable for a successful separation of microchip electrophoresis with plastic microchips. Because of the great variance of the bulk composition and the surface properties of different plastics, a great number of surface modification protocols have been proposed [7–9].

Cyclic olefin copolymer (COC) is a thermoplastic polymer from cyclic olefin monomers and ethylene [10], which with merits such as high transmittance in UV spectral region, low fluorescence background, low permeability, high mechanical strength, easy molding,

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high organic solvent resistance, minimum water adsorption, and good biocompatibility [11]. A main drawbacks of this material is its poor hydrophilicity. To improve its hydrophilicity, Lee et al. developed a UV-mediated grafting technique for double-stranded DNA separation [12] and isoelectric focusing of proteins in COC mcirochips [13], Luna-Vera et al. modified COC micro-channels with photografting of polyethylene glycol acrylate (PEGA) for a real-time detection of Lysozyme [14]. All these protocols work very well but the modification itself adds extra steps of the analysis and compromises the cost advantage of the COC microchips. Dynamic coating with polymers such as linear polyacrylamide (LPA) [15], polyethylene oxider (PEO) [16], hydroxyethyl cellulose (HEC) [17], hydroxypropyl cellulose (HPC) [18], polyvinyl alcohol (PVA) [19] etc. and surfactants such as didodecyldimethylammonium bromide (DDAB) [20], Triton X-100 [21], sodium dodecyl sulfate (SDS) [22] can be another way to suppress surface charge and eliminate non-specific adsorption, which is apparently potential to achieve the surface modulation without extra burden of microchip preparation.

Rhodamine dyes normally show bright color and have been widely used in industry and research laboratories. However, they are not allowed to be used in food due to their potential toxicity. Rapid detection of these dyes is necessary for food quality control and other related areas. Rhodamine dyes are normally fluorescent, can be easily detected using fluorometry, but separation based techniques have to be used for the determination of individual rhodamine dyes. Successful determination of rhodamine dyes through microchip electrophoresis have been reported. Kang et al. performed separation and detection of rhodamine dyes using the non-ionic surfactant Triton X-100 in poly (dimethylsiloxane) (PDMS)/glass hybrid chip [21]. DDAB was used by the same group to reduce the fluorescent dyes adsorption onto PDMS microchannels [20]. Culbertson's [22] group added SDS into the background electrolyte (BGE) and reduced the adsorption of rhodamine dyes through the interaction between SDS and rhodamine, satisfactory separation of rhodamines was achieved. Despite these successes, we found that it was hard to perform the separation of these rhodamine dyes with surfactants in COC microchips with relative larger microchannels due to the strong adsorption of the dyes on the COC surface [23].

Adsorption problem can be solved by non-aqueous capillary electrophoresis [24]. Fakhari et al. successfully separated five hydrophobic basic blue dyes [25] with this technique, and Pelaez-Cid et al. determined eight textile dyes through non-aqueous capillary electrophoresis with electrochemical detection [26]. Nuchtavorn et al. separated four blue dyes on microchips using organic solvent [27]. Apparently, organic solvents play an important role for reducing the adsorption of these analytes. In the present work, high content of organic solvent (80%) was used in BGE to offer a similar organic environment to NACE and polyacrylic acid (PAA) was used as a multifunctional additive in microchip electrophoresis for the separation of rhodamine dyes in COC microchips. PAA was selected as a dynamic surface modifier because PAA is an ionic polymer, which is soluble both in water and many orgainc solvents, and can regulate the viscosity of the BGE. Its ionic nature together with high content of organic solvent should be beneficial to eliminate the analyte adsorption caused by electrostatic attraction and hydrophobic interactions. Despite these advantages of PAA, it has been rarely used as the additive of capillary or microchip electrophoresis except the application of its complexes with cationic surfactant as pseudo-stationary phases in micellar electrokinetic chromatography [28,29]. This work attempts to confirm the potential of polyelectrolytes as multifunctional additives for highly efficient separation of MCE with BGE containing high content of organic solvent on plastic microchips.

2. Experimental

2.1. Apparatus

The laser-induced fluorescence (LIF) detector used in the present work was constructed in our laboratory and has been described previously [30]. Main components include a DPSS laser (473 nm, MBL-III-473, Changchun New Industries Optoelectronics Tech Co. Ltd., Changchun, China), a filter set containing a dichroic mirror (505 nm, DM505, Shenyang HB Optical Technology Co. Ltd., Shenyang, China) and a long-pass filter (520 nm, BP520, Shenyang HB Optical Technology Co. Ltd., Shenyang, China), and an avalanche photodiode (APD, AD500-8-TO52S2, Silicon Sensor, German). The detector was arranged in a confocal configuration and the fluorescence signal was fed to a NI USB-6009 data acquisition card (National Instruments, Austin, TX, USA) for electropherogram recording through a program written in Labview (National Instruments).

The high-voltage power supply comprises a high-voltage module (DW-P602-200F, Dongwen High-Voltage Power Supply, Tianjin, China), and an electronic circuit to drive solenoid valves and measure the current of the electrophoresis. A three-way solenoid valve (WTB-3R-N4F, 12 VDC, Takasago Electric Co. Ltd., Suzhou, China) was used to switch the negative pressure applied to the sample waste reservoir on a microchip for the negative pressure induced injection of samples. The output of the high voltage, switching of the solenoid valve were controlled through the same USB-6009 with the aforementioned program used for electropherogram acquisition. Negative pressure was provided by a reversely connected aquarium air pump (SB-2800, Sobo electric appliance Co. Ltd, Zhongshan, China).

2.2. Device fabrication and microchip electrophoresis procedure

All microchips were fabricated using a wire-embossing method [31] as described previously [32]. The microchips possess microchannels of 75 ± 5 μm microchannels arranged in a simple cross pattern. The separation channel was totally 4.5 cm long and 3.5 cm to the cross point. The sampling channel was 1.2 cm long and 0.6 cm to the cross point. The four reservoirs were named following the nomenclature as B, BW, S and SW, which represent Buffer and Buffer Waste at the both ends of the separation channel, and Sample and Sample Waste at the ends of the sampling channel. Negative pressure pinched sample injection [33] was used in the microchip electrophoresis as described previously [30]. Sample was driven to the cross point by the negative pressure and forced to the separation channel by the high voltage immediately applied across the separation channel. Leakage of the sample into the separation channel was suppressed by lower liquid levels in both S and SW reservoirs. The solution normally added into the four reservoirs were 170, 70, 15 and 0 μL in B, BW, S and SW, respectively, to ensure successful sample injection and leakage suppression during the separation.

2.3. Chemicals

Polyacrylic acid (molecular weight: 4.5×10^5) was purchased from Aladdin Chemistry Co. Ltd. (Shanghai, China). Sodium dihydrogen phosphate was a product of Tianjin Guangfu Fine Chemical Research Institute (Tianjin, China). Rhodamine 6G was a product of Merck. Rhodamine B was purchased from Chengdu Kelong Chemical Reagent Factory (Sichuan, China). Anhydrous methanol was a product of Tianjin Baishi Chemical Co. Ltd. (Tianjin, China). All reagents were analytical grade and used as received. Chilli powder and lipsticks were obtained at local markets.