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Ionic polymer enhanced electrophoresis in plastic microchips for rapid and robust determination of rhodamine dyes

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ABSTRACT

Electrophoresis on plastic microchips is an economic way for rapid separation and determination of multiple analytes, but its application is frequently limited by the unstable electroosmotic flow (EOF) and non-specific adsorption of certain analytes and sample matrices. In this work, cationic polymer poly (diallyldimethylammonium chloride) (PDDA) was used as the background electrolyte (BGE) to eliminate the non-specific adsorption, control the EOF, regulate the viscosity and improve the separation efficiency on cyclic olefin copolymer microchips with 75 μm id microchannels. Three rhodamine dyes that are easily adsorbed on plastics were separated in the presence of organic solvents. Influences of the experimental conditions were systematically investigated and the performance was compared with anionic polymer polyacrylic acid or neutral polymer hydroxypropyl cellulose. The effectiveness of ionic polymers for the modulation of EOF was demonstrated. With PDDA as BGE, three rhodamine dyes were well separated and baseline separation could be achieved in 20 s with a voltage as low as 400 V using a microchannel of 1 cm long and 75 μm id. The determination of rhodamine dyes in foods and lipsticks was performed to verify the robustness and applicability of the proposed methods. Separation of peptides and proteins labeled by rhodamine B isothiocyanate was also performed with the proposed running medium.

1. Introduction

Rhodamine is a group of fluorescent dyes with a main frame of xanthene. Because of their excellent photophysical and photochemical properties, these dyes have been widely used in biochemical analysis, environmental chemistry and optical science [1–3]. They are also commonly employed in the textile, printing and photographic industries [4,5]. However, there are evidences to show that the rhodamine dyes can stimulate the skin, eyes and respiratory tracts, even have potential toxicity and carcinogenicity to human body [6,7]. They are prohibited to be used in foods and regulated as cosmetic additives in many countries and regions [8–10]. However, illegal use of these dyes can still be found frequently because of their wide availability and high effectiveness. Therefore, a rapid, sensitive and efficient method to detect rhodamine dyes is necessary for the supervision of these products.

Although there are a variety of analysis techniques available for the determination of rhodamines, such as ultraviolet-visible spectrophotometry [11], fluorescence spectrophotometry [12], electrochemiluminescence [13] and electrochemical methods [14], these techniques can hardly realize simultaneous determination of these dyes because of the overlap of their spectra [15], especially when they present in trace amounts with complicated sample matrices [16]. To improve the specificity, Song et al. [17] prepared a polyclonal antibody of rhodamine B (RhB) using white rabbits and established a method combining enzyme-linked immunosorbent assay and immune affinity column for the detection of RhB. Separation-based methods such as high performance liquid chromatography (HPLC) [18] and capillary electrophoresis (CE) [16] have been used to analyze rhodamine dyes in practical samples. These methods are selective but they normally need complicated and expensive devices.

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Microchip electrophoresis (MCE) has been proved to be a powerful platform for rapid analysis of rhodamine dyes. Jacobson et al. [19] have shown that RhB and dichlorofluorescein can be separated within 0.8 ms. However, due to the relatively high cost of the glass microchips, real application of this technique is still limited. To reduce the cost of microchips, plastics have been widely used and there are plenty of reports confirmed the advantages of these materials [20]. But there are other factors that restrain their wide application in the real analysis, for example, surface modification that is frequently needed for plastic microchips can largely compromise their cost advantage, while randomly happened clogging and contamination of microchannels make this technique highly skill-dependent and slow down its pace toward the real-world analysis. Due to the hydrophobic surface of most plastic materials, the adsorption of biological macromolecules and some small organic molecules onto the surface of microchannels may cause unstable electroosmotic flow (EOF) and poor analysis performance [21,22]. Microchannel clogging caused by the air-borne particles can ruin the separation too, a clean operation area is important for reproducible separation. To improve the robustness of MCE, we adopted water-soluble polymers as multifunctional additives or components to 1) eliminate the adsorption of sample components, 2) maintain a suitable viscosity to decrease the effect of pressurized flow caused by unbalanced liquid levels and variation of EOF along the microchannel, 3) ensure high separation efficiency in relative large microchannels to reduce the chance of clogging [23–26].

The addition of polymers as additive for dynamic surface modification is a common practice in CE, ionic polymers were also used as pseudostationary phase in CE or capillary electrochromatography (CEC) [27–29] and as sieving matrices [30]. But the contribution of polymers to the viscosity of the background electrolyte (BGE) is not critical because of the long capillary (e.g. 50 cm) used in normal CE and CEC. Successful implementation of electrophoretic separation in microchips normally requires much thinner or narrower microchannels due to their shorter lengths, which significantly deteriorates the robustness of the technique. Higher viscosity is therefore critical if larger microchannels are used to avoid clogging and facilitate the liquid filling. These factors make MCE not a simply miniaturized version of CE or CEC, different protocols should be established for reliable and efficient separation with MCE.

There are some successful separations of rhodamine dyes in polymeric microchips with surfactants as the additives. Wang et al. [31] reduced the adsorption of RhB on poly (dimethylsiloxane) (PDMS)/glass composite microchip effectively by adding nonionic surfactant Triton X-100 in BGE. It was found that the ionic liquid could enhance the inhibition ability of Triton X-100 for RhB adsorption [32], and the double chain cationic surfactant alkyl dimethyl ammonium bromide could get rid of the adsorption of pyronine Y and RhB in PDMS microchip [33]. The RhB and other hydrophobic molecules were separated successfully by micellar electrokinetic chromatography (MEKC) in PDMS microchips in the presence of sodium dodecyl sulfate (SDS) [34]. However, the depths of microchannels are no larger than 20 μm in these works.

The poor compatibility of these surfactants mentioned above with organic solvents may be another concern due to the effect of organic solvents on the micelles stability [27,28,35], while organic solvents are indispensable for the separation of hydrophobic analytes [36–38]. Polymers may have much higher compatibility with organic solvents and can be used for surface passivation and viscosity regulation. The last one is particularly important for reducing the clogging chance through relatively larger microchannels.

In the present work, poly (diallyldimethylammonium chloride) (PDDA) was used as a main component of BGE together with organic solvents to realize the efficient and robust separation of rhodamines dyes in the absence of other buffer. A systematic comparison with anionic polyacrylic acid (PAA) and neutral hydroxypropyl cellulose (HPC) was performed to illustrate the roles of these polymers and their interaction with analytes and microchannel surface. The aim of the work is to establish a general strategy for MCE with hydrophobic surfaces to control EOF, eliminate unnecessary adsorption of analytes and modulate interaction between analytes and BGE components through proper selection of polymers.

2. Experimental

2.1. Materials and chemicals

RhB was purchased from Chengdu Kelong Chemical Reagent Factory (Chengdu, China). Rhodamine 6G (Rh6G) was a product of Merck. Rhodamine 123 (Rh123), PDDA (Mw 400,000–500,000, 20 wt.% aqueous solution), PAA (Mw 450,000) and rhodamine B isothiocyanate (RBITC, mixture of isomers) were purchased from Aladdin Reagent Co., Ltd. (Shanghai, China). HPC (reagent grade) was purchased from Sangon Biotech Co., Ltd. (Shanghai, China). Methanol, ethanol, acetone, acetonitrile, dimethyl sulfoxide (DMSO) and sodium hydrogen carbonate were products of Rionlon Bohua Pharmaceutical & Chemical Co., Ltd. (Tianjin, China). Isopropanol was obtained from Tianjin Chemical Reagent Co., Ltd. (Tianjin, China). Anhydrous disodium hydrogen phosphate, sodium dihydrogen phosphate and potassium dihydrogen phosphate were products of Tianjin Guangfu Fine Chemical Research Institute (Tianjin, China). Sodium hydroxide, sodium chloride and pyridine were bought from Tianjin Guangfu Science and Technology Development Co., Ltd. (Tianjin, China). Hydrochloric acid was from Beijing Chemical Factory (Beijing, China). Catchup, chili powder and lipsticks were purchased from local markets. Thymopentin (≥97%) was a product of Kaibo Pharmaceutical Co., Ltd. (Lanzhou, China). Ribonuclease A (RNase A) from bovine pancreas (80%) was bought from Sigma-Aldrich (St. Louis, MO, USA). Lysozyme was purchased from Beijing BioDee BioTech Co., Ltd. (Beijing, China). All chemicals were analytical grade except indicated otherwise and used as received without further purification. Distilled water was used in all experiments. All solutions were filtered through 0.45 μm membranes (Shanghai Xinya Purification Materials Factory, Shanghai, China) before use.

2.2. Apparatus

The laboratory-made instruments used for MCE have been described previously [25]. The high voltage and a 3-way solenoidal valve for switching the negative pressure were controlled through a program written in LabVIEW (National Instruments), which is also used for electrophoretic current and electropherogram acquisition. The viscosity of the solution was measured by an Ubbelohde viscometer (inner diameter: 0.8–0.9 mm, viscometer constant: 0.05123 mm^2/S^2, Shanghai Shenyi Glass Products Co., Ltd., Shanghai, China) in a super thermostatic water bath (DF-02, Nanjing Fangao Technology Co., Ltd., Nanjing, China) set at 25 °C. A conductivity meter (DDS-307, Shanghai Precision Scientific Instrument Co., Ltd., Shanghai, China) was used to measure the conductivity of the prepared solution.

2.3. Sample preparation

Rhodamine dyes in food samples were extracted with methanol. Typically, 5 mL of anhydrous methanol were added to 0.5 g accurately weighted catchup or chili powder in a 10 mL centrifuge tube. The mixtures were placed in an ultrasonic bath for 10 min to extract dyes. For the lipsticks, 25 mg of cream was accurately weighed and directly dissolved in 5 mL anhydrous methanol in a 10 mL