



Electrokinetic stacking of electrically neutral analytes with paper-based analytical device

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ABSTRACT

Electrokinetic stacking (ES) is effective for improving sensitivity of paper-based analytical device (PAD) for charged analytes. In this paper, we successfully demonstrated ES of electrically neutral analytes on PAD, and the performance was characterized by smartphone-based colorimetry and fluorescence. Firstly, SDS from cathode reservoir stacked as a micelle band on an open paper fluidic channel by ES, and the target analyte was swept by the micelle. Meanwhile, the probes at the other side were carried by electroosmotic flow (EOF). Eventually, neutral components preloaded on the channel were concentrated as the narrow stacking band. Taking the rhodamine B as a probe, the effects of EOF, background electrolyte concentration and anionic surfactant concentration were investigated. Fluorescence detection of rhodamine B and colorimetric analysis of Sudan III demonstrated the sensitivity enhanced and its potential for the semi-quantitative test. Under the optimized conditions, fluorescence detection limit of 50 nM of rhodamine B was achieved with a linear range of 1.0–10 μM ($R^2 = 0.99$). The colorimetric detection limit for Sudan III was 5.2 μM and the linear range was 5–40 μM ($R^2 = 0.99$). Compared with direct analysis without stacking, the signal levels of rhodamine B and Sudan III were increased by 30-fold and 6-fold, respectively. This study showed that with ES, sensitive and rapid PAD detection of electrically neutral analytes could be achieved.

1. Introduction

Paper-based analytical device (PAD) as an on-site detection platform is suitable for environmental monitoring, food quality control and clinical diagnostic due to its low cost, simplicity of fabrication, compatibility with other detection methods and so on [1–3]. The enhancement of detection sensitivity is particularly significant for further widening the application scope of PAD. In addition to the introduction of sensitive detection methods [4], signal amplification or sample preconcentration methods such as biological or chemical signal amplification [5–8], solvent evaporation [9], electrokinetic stacking (ES) (isotachopheresis (ITP), ion concentration polarization (ICP), field-amplified sample stacking (FASS)) [10–17] are also effective for reducing the detection limit. ES is commonly used in capillary electrophoresis [18] and it also shows good stacking effect in PAD. Ma et al. achieved 1000-fold of λDNA within 300 s in PAD combined with FASS [14]. Under normal conditions, ES is only used for the stacking of charged components [15]. In addition to charged analytes, on-site detection of electrically neutral analytes, such as steroids (progesterone, cortisol, prednisone), organophosphorus pesticides (parathion,

fenitrothion, pyriithione), monosaccharides, nucleosides, aflatoxin B1, Sudan, flavins, are also significant for food safety, environmental testing and disease diagnosis [19–23]. Sweeping was commonly used to online enrich neutral molecules in capillary electrophoresis [24,25], which was firstly proposed by Quirino and Terabe in 1998 [26]. The sweeping mechanism is based on the interaction of analytes with micelles, and micelles sweeping can be implemented in two ways [27]. One is in homogeneous electric field, in which analytes are prepared in the solution with the same conductivity as the BGE, and the neutral analytes are swept by the micelles and form a narrow concentration zone. Noticeably, the used micelle concentration is greater than its critical micelle concentration (CMC). Based on this method, Vitamin B was concentrated by 40-fold [28], and the sensitivity of enrofloxacin and ciprofloxacin was improved 376 and 406-fold, respectively [29]. Another mode is in heterogeneous electric field, in which the micelles are concentrated at the interface between the high-conductive sample zone and the low-conductivity background electrolyte zone. The analyte at one end of the sample region is picked up and accumulated by the accumulated micelles and other analyte migrates into the micelle zone by electroosmotic flow. Bocek et al. achieved nearly 10-fold

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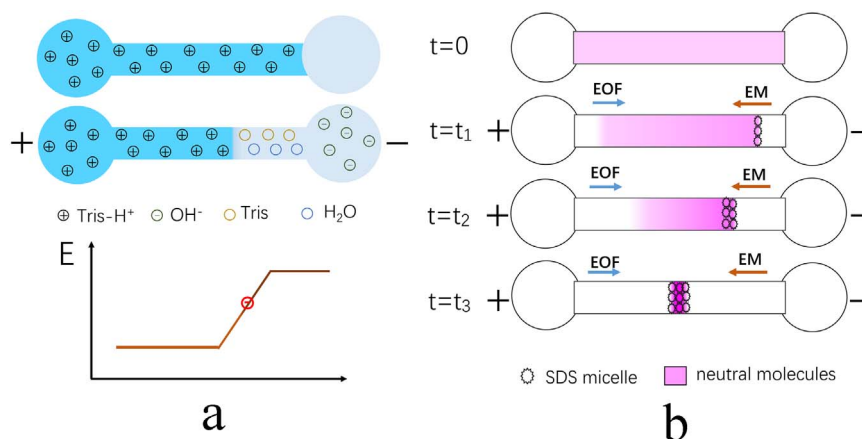


Fig. 1. (a) The formation of electric field gradient. (b) The mechanism of neutral molecules stacking. EOF was electroosmotic flow. EM was electro migration.

concentration of alkyl phenyl ketones and progesterone with this method [30]. To date, no work on electrokinetic stacking of neutral analytes in PAD has been found in the literature.

Based on the principle of analytes stacking in the heterogeneous electric field mentioned in capillary electrophoresis and our investigation on electrokinetic stacking of charged components, we proposed a method for electrokinetic stacking of neutral analytes on PAD for the first time. The mechanism is schematically shown in Fig. 1. Firstly, an electric field gradient is established on a paper fluidic channel based on the titration reaction (Fig. 1a) [15]. Secondly, the negatively charged anionic surfactants from cathode side stack on the electric field gradient and form micelles that sweep the neutral analyte preloaded on the paper channel into the stacking band. Simultaneously, the target analytes on the anode side are transported to the stacked micelles band by electroosmotic flow (EOF). Eventually, an integrated stacking band is formed as shown in Fig. 1b.

The feasibility of the proposed method was successfully demonstrated by using fluorescent rhodamine B as the visual probe. 30-fold signal enhancement was achieved under optimal conditions, as shown by a PAD system with LED excited fluorescence and smartphone imaging. The detection of Sudan III was also shown by smartphone-based colorimetric analysis, demonstrating its potential application in rapid on-site detection of illegal dye additives in food.

2. Experimental

2.1. Materials and reagents

Trihydroxymethylaminomethane (Tris), sodium dodecyl sulphate (SDS), hydroxyethyl cellulose (HEC), cetyl trimethyl ammonium bromide (CTAB), hydrochloric acid (HCl) were purchased from Lab Science and Trade Co., Ltd. (Shenyang, China). Rhodamine B (Aladdin Chemical Co., Ltd., Shanghai, China), a common electroosmotic flow marker [31], was dissolved in the background electrolyte (BGE). Sudan III (Shandong West Asia Chemical Co., Ltd., Linyi, China), toxic non-ionic lipophilic substance, was dissolved in methanol [32]. Above reagents were of analytical grade and used without further purification. Glass fiber filter (K-49, Kejia Environmental product Co., Dezhou, China) was used as the paper substance, which was cut into the strips (35 mm × 3 mm) with a cutter machine (No. 8061, Deli Stationery, Ningbo, China). Pt electrodes, the diameter of 0.5 mm, were placed in a cap of 1.5 mL plastic centrifuge tubes as the reservoir.

2.2. Instrumental

The experimental setup was shown in the Supplementary material (Fig. S1). The power source of 300 V was composed of portable power

supply (output voltage of 5 V) and a voltage converter (GRB12300D, ESSO Latham Electronic Technology Co., Ltd., Shenzhen, China) that converted 5 V to stable 300 V. A multimeter was used to monitor the current variation during the stacking process. Green light-emitting diode (LED, 520 nm ~525 nm), white LED (6000–6500 K) were obtained from Xin Huakai Photoelectric Co., Ltd. (Shenzhen, China) and used as the fluorescence excitation light source for rhodamine B probe and lighting source for Sudan III probe, respectively. The smartphone was installed either in the stereomicroscope (ZTX-3E-3C, Huaguang Precision Instrument Co., LTD., Ningbo, China) containing long pass filter (500 nm ~700 nm) or connected with a macro lens (Mai Pu Luo Technology Co., Ltd., Shenzhen, China) to compensate the focusing capability of the camera. In the quantitative analysis of Sudan III, smartphone with macro lens was used to demonstrate the portability of the PAD system for on-site analysis.

2.3. Device operation

The paper channel was placed on the two reservoirs fixed by double-side adhesive tape, with a distance of 25 mm. 40 μL sample solutions were uniformly loaded onto the paper channel by pipette. Afterwards, 200 μL Tris-HCl (pH 8.1, 25–100 mM), SDS solution (1–10 mM) diluted with deionized water were put into the anode reservoir and cathode reservoir, respectively. Under the application of a DC voltage, the neutral probes were automatically stacked into a narrow band on the paper channel based on the dual effect of the sweeping of micelles and electroosmotic flow. The band image was recorded by the smartphone. Within the imaging process, a shade cloth was used to cover the system to avoid the influence of ambient light conditions.

2.4. Imaging processing

The smartphone parameters (white balance, focusing mode, metering mode, ISO, exposure compensation) were controlled with a Camera FV 5 software. The images acquired by the smartphone were stored in JPG format and processed by Image J (National Institutes of Health, USA). The difference of gray intensities between the detection zone and background on the same paper channel was the actual colorimetric intensity of the sample stacking band.

3. Results and discussion

3.1. Proof of concept

According to the reported investigation of ES mechanism on PAD [15], we could speculate that SDS as an anionic component could be stacked on the established electric field gradient. Using the neutral

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