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Two dimensional separations of human urinary protein digest using a droplet-interfaced platform

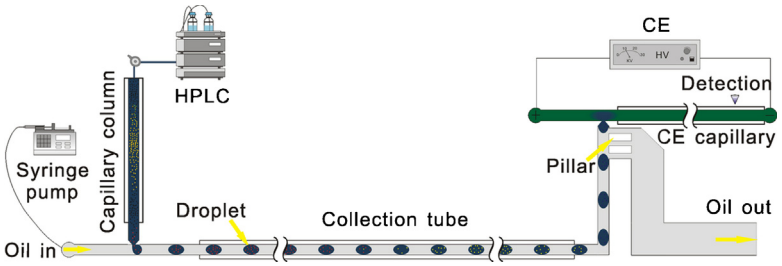
Linquan Ye [a], Xin Wang [a], Jing Han [a], Fan Gao [a], Lingjia Xu [a], Zhiliang Xiao [a], Peiming Bai [b], Qiuquan Wang [a], Bo Zhang [a,*]

[a] Department of Chemistry and The MOE Key Laboratory of Spectrochemical Analysis and Instrumentation, College of Chemistry and Chemical Engineering, Xiamen University, Xiamen 361005, China
[b] Department of Urology, Xiamen University Affiliated Zhongshan Hospital, Xiamen 361004, China

HIGHLIGHTS

- Droplet microfluidics was used as a nanoliter sample transfer tool for 2D LC–CE.
- High resolution profiling of a human urinary protein digest was realized.
- The droplet-interface ensured 100% non-loss sample transfer.
- Fine fractionation capability and sampling completeness was demonstrated.
- Multiplex use of the strategy can enable super high resolution (bio) separations.

GRAPHICAL ABSTRACT



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ABSTRACT

For highly complex mixtures, coelution is a common phenomenon in chromatography. A great deal of resolution is hidden in coelution, and lost due to inevitable molecular diffusion during sample transfer. The molecular diffusion may lead to band broadening and remix of separated peaks, which cause degradation of achievable resolution. In this study, we introduced droplet microfluidics as a high performance sample transfer tool in two dimensional nanoflow liquid chromatography–capillary electrophoresis separation of a human urine sample. The fine fractionation capability and sampling completeness enabled by the droplet-interface demonstrated the 2D system's usefulness in high-resolution mapping of real world biological samples.

1. Introduction

Proteomics has become one of the top challenges for life science and analytical technologies following the successful completion of human genome project [1]. Due to the high complexity of proteomic samples, e.g., human plasma proteome contains over 50,000 proteins spanning over 10 orders of magnitude in abundance [2], multidimensional separation techniques with high resolution, such as two dimensional gel electrophoresis (2D GE) and two dimensional liquid chromatography (2D LC), have been widely used in today's proteomics laboratories [3].

For the coupling of two separation dimensions, the core issue is the interface, the device through which pre-separated samples from the first dimension (1st D) being transferred to the second

* Corresponding author. Tel.: +86 592 2188691; fax: +86 592 2188691.
E-mail address: bozhang@xmu.edu.cn (B. Zhang).

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separation dimension (2nd D), ideally, without sacrificing the resolution achieved in 1st D [4]. Conventionally used 2D liquid phase separation strategies transfer samples between dimensions in a continuous flow manner [5]. In general, the risk of resolution loss in a 2D interface is reflected in three aspects: (1) *band broadening*, since molecular diffusion is always taking place, long sample transfer time and/or flow path may lead to peak dispersion and result in less efficient peaks [6]; (2) *remix*, when adjacent peaks experience such molecular diffusion, the already separated peaks in 1st D may merge back during sample transfer; (3) *fractionation frequency*, according to the theoretical work [7], a peak should be sampled at least three times in order to retain its resolution in transferring from 1st to 2nd dimension. Therefore, high frequency fractionation of sample peaks is preferred. Moreover, real-world proteomic samples may have only limited amount available for analysis, e.g., diseased tissue or even single tumor cells. Therefore, multidimensional separations based on microcolumn techniques [8,9], such as nanoflow liquid chromatography (nanoLC) and capillary electrophoresis (CE), have become the method of choice, especially in the case when coupled with mass spectrometer (MS). Consequently, high performance interface technology which can support nanoliter fluid manipulation and high resolution and non-loss sample transfer is in great demand for proteomic separations.

Droplet microfluidics uses two immiscible phases, e.g., aqueous and oil, to generate and manipulate discontinuous fluid flow [10,11]. It enables diffusion-free operation of extremely small volumes of liquid, such as micro- to nanoliter and even down to femtoliter [12,13]. From a separation science point of view [14], droplet microfluidics has multiple advantages in securing high performance sample transfer. Using droplet microfluidics, continuous flow of liquid phase separations, such as HPLC and CE, can be converted to discontinuous droplet flow with a finely controllable resolution [15–23]. In this way, following a separation, analytes distributed spatially and temporally in the continuous effluent flow can be compartmentalized into a series of discrete droplet fractions, with their spatiotemporal resolution well maintained. Therefore, the abovementioned issues, such as band dispersion and remix may be greatly diminished. Meanwhile, due to the flexibility of droplet microfluidics, the size of droplet fractions, as a result of segmentation frequency, can be controlled at tiny volumes (e.g., nL). Hence, the fractionation fineness of sample peaks in 2D separations may be maintained at a high resolution level. In this study, using a droplet microfluidic interface for 2D nanoLC–CE coupling, we investigate the 2D system's practical robustness and resolving power for a real-world human urine proteomic sample.

2. Experimental

2.1. Materials and apparatus

Trifluoroacetic acid (TFA), dithiothreitol (DTT), iodoacetamide (IAA), ammonium bicarbonate, acetone, sodium dihydrogen phosphate, phosphoric acid, formic acid, urea, thiourea, albumin from bovine serum (BSA) and proteomics grade trypsin (from porcine pancreas) were purchased from Sigma–Aldrich (St. Louis, MO). HPLC grade acetonitrile (ACN) was obtained from Merck (Darmstadt, Germany). Fluorinert Engineered Fluid FC-40 [24] (Part number ZF-0002-1308-0), used as the oil phase in droplet microfluidics, was purchased from 3M (St. Paul, MN). Three polypeptides: angiotensin acetate (Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu), somatostatin acetate (Ala-Gly-Cys-Lys-Asn-Phe-Phe-Trp-Lys-Thr-Phe-Thr-Ser-Cys disulfide bridge: 3–14) and oxytocin (Cys-Tyr-Ile-Gln-Asn-Cys-Pro-Leu-Gly disulfide bridge: 1–6) were synthesized and purified by Sangon Biotech (Shanghai, China) with purity greater than 98.0%. Ultrapure water (18.2 MΩ) was prepared in a Milli-Q system (Millipore, Bedford, MA) and used throughout this study. All chemicals and reagents were at least of analytical or high grade and used without further purification. Fused silica capillaries, 100 μm i.d., 365 μm o.d., were purchased from Yongnian Reafine Chromatography (Hebei, China). Reversed phase material, Ultimate XB-C18, 5 μm, 300 Å, from Welch Materials (Shanghai, China) was used for capillary column packing. An Elite P230 high pressure pump from Dalian Elite Analytical Instruments (Dalian, China) was used for column packing. Portex fine bore polyethylene tubing (0.38 mm i.d., 1.09 mm o.d.) used for

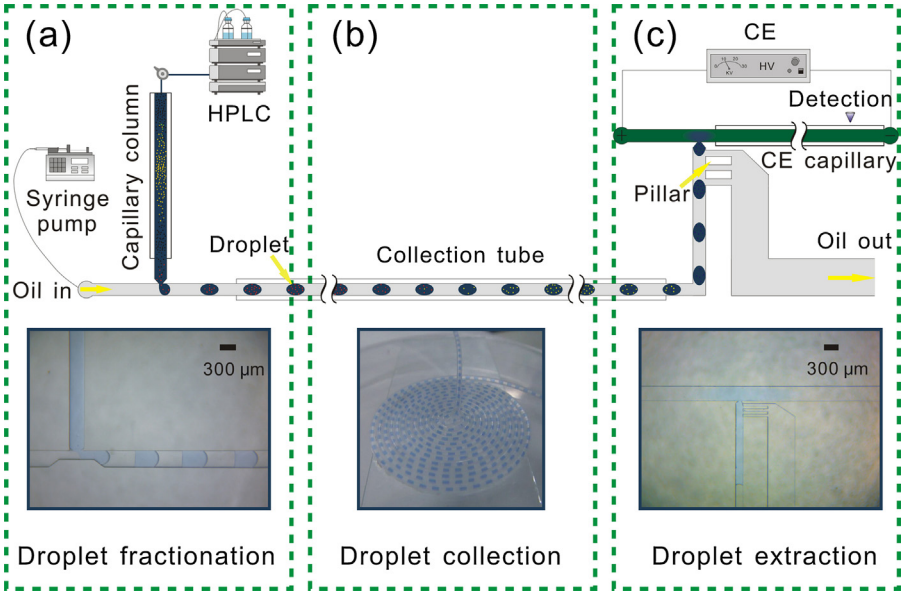

Fig. 1. A droplet-interfaced 2D nanoLC–CE system. HPLC effluent was fractionated into a series of nanoliter droplet units right after chromatography (panel a), and collected and stored in a tube (panel b, tube i.d. 0.38 mm), before drop-wise analyzed in CE (panel c). Photographs for each stage of the workflow are shown at the bottom. Blue ink was used to represent aqueous droplets, which were spaced by oil phase. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)