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A stable and high-resolution isoelectric focusing capillary array device for micropreparative separation of proteins

Si Li [a,1], Jing-Yu Dong [a,b,1], Chen-Gang Guo [a], Yi-Xin Wu [c], Wei Zhang [a], Liu-yin Fan [a], Cheng-Xi Cao [a,*], Wei-Bing Zhang [b,**]

[a] Key Laboratory of Microbial Metabolism of Education Ministry, School of Life Science and Biotechnology, Shanghai Jiao Tong University, Shanghai 200240, China
[b] School of Chemistry and Molecular Engineering, East Chinese University of Science and Technology, Shanghai 200237, China
[c] Ruijin Hospital, Medical School, Shanghai Jiao Tong University, Shanghai 200240, China

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ABSTRACT

A simple capillary array IEF device was developed for high resolution and micropreparative separation of trace amounts of proteins. Based on quasi-chip-scale manufacturing, the specific capillaries (600 μm i.d., 1200 μm o.d. and 20 mm length) were integrated with the miniaturized polymethyl-methacrylate electrode trays. Electroosmotic flow was suppressed effectively by modified cross-linked polyacrylamide coating, and instability of IEF was addressed using the designed concentration of electrolytes via moving reaction boundary theory. As a prototyping, the resolution, reproducibility, throughput, speed and linearity of pH gradient were systemically evaluated with model proteins. The results revealed the following advantages: (i) the reproducibility of array was assessed as RSD values of 0.95% (intra-day) and 2.88% (inter-day); (ii) IEF could be completed in 20 min with up to 400 V/cm electric field; (iii) high resolution separation of model proteins achieved in 20 mm length column; (iv) multi-units with 48 micro-columns can be easily integrated to obtain high throughput; and (v) good linearity of pH gradient (R=0.9989). More importantly, utility of the device was tested by using hemoglobins sample from human red blood cell. HbA_0 and HbA_{1c} with only ΔpI 0.03 have been successfully separated by the developed method.

1. Introduction

The idea of isoelectric spectra, viz., the prototype of isoelectric focusing (IEF), was unveiled by Kolin [1], and yet the separation of protein and virus in line with their p*I*s could only last for about 10 min due to the lack of carrier ampholyte (CA). Svensson [2] advanced the theory of classic IEF and pointed out the crucial role of CA in IEF. Accordingly, Vesterberg [3,4] successfully synthesized CA in accordance with the classic theory, leading to the real application of gel-based IEF in biological research. As the first dimension separation in 2DE, the classic gel IEF has been used extensively in the analyses of complex protein samples [5,6]. Nevertheless, there were still a few of issues that weaken the separation performance of the IEF, such as instability of pH gradient, unsatisfactory separation of proteins with minor p*I* difference (e.g., hemoglobin A and hemoglobin A_{1c}) and time-consuming (e.g., up to 10-h run of gel IEF) [7,8].

The technique of capillary IEF (cIEF), initially reported by Hjertén et al. [9] with a salt-induced mobilization step, made significant advances in improving the resolution via high electric field thanks to the high specific surface area. The complex peptide mixture such as the lysate from yeast cytosol could be separated by cIEF in 10 min [10]. To address the problem of throughput, a series of array cIEF systems that coupled with liquid chromatography (LC) were developed for the analyses of multiple protein samples [11,12]. Remarkably, the array cIEF device with up to 60 coated capillaries was reported by Mao et al. [13]. A reagent-release capillary array device was developed by Kataoka et al., by which the mixture of standard hemoglobin AFSC have been separated [14]. The array prototype of capillary shows a promising manner for research of complex proteins by integrating high resolution and throughput. Nevertheless, the pretreatment process of traditional capillary was time-consuming. Besides, the repeated rinse between inter-assays will surely weaken the reproducibility of proteins separation.

Numerous chip-based IEF approaches that required extremely small sample volume have been proposed for the rapid separation

* Corresponding author. Tel./fax: +86 21 3420 5820.
** Corresponding author.
E-mail addresses: cxcao@sjtu.edu.cn (C.-X. Cao), weibingzhang@ecust.edu.cn (W.-B. Zhang).
[1] The first two authors have equal contribution to the work.

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of proteins [15–20]. Dauriac et al. [16] described a $5 \times 3.9\ cm^2$ array micropillar device and displayed the rapid IEF of seven visible proteins. Herr et al. [17] developed an acrylic microfluidic chip device, in which the separation time of three fluorescent proteins was less than 1 h. Zilberstein et al. [18] reported the parallel IEF microdevice using a dielectric membrane with conducting channels for protein separation in several minutes. The mobilization step in cIEF that resulted in band distortion and resolution damage could be removed by whole-column imaging strategy in chip-based IEF. For instance, Pawliszyn's group [19,20] reported the special work that was aimed at the miniaturized UV detection device and hybrid materials construction. However, the resolving power achieved by chip-based IEF was barely satisfactory. For example, the complete separation between normal hemoglobin and the glycated species was not conducted in chip-based IEF yet [21].

The array cIEF and chip-based IEF respectively have its own focus on protein research. In this work, we endeavored to combine the advantages of the two strategies for the high resolution and throughput separation of trace amount of proteins such as glycated hemoglobins. The prototyping and manufacturing of the miniaturized device was described. Briefly, the specific capillary (600 μm i.d., 1200 μm o.d. and 20 mm length) with no outer polymer coating was employed as the disposable separation channel. The designed electrolytes pair by the moving reaction boundary theory (MRB) [7], and the modified cross-linked polyacrylamide (CPA) coating were applied to generate stable pH gradient. The separation performance of the device such as throughput (up to 48 parallel runs), stability and resolution were systematically evaluated. Notably, HbA_0 and HbA_{1c} with only 0.03 p*I* difference from human blood erythrocytes were separated in 20 min with high resolution, which has not been reported in chip-based IEF research. In addition, a very rare of the phenomenon that the standard cytochrome (Cyt) C consistently presented two focused bands in IEF was shown, and a possible mechanism was discussed. The developed device was further applied for the micropreparative separation and identification of Hb species from diabetics in our accompany work [22]. Additionally, the comparison of resolution between developed device and traditional IEF method was discussed, including large-scale column and immobilized pH gradient strip (IPG) IEF.

2. Experimental section

2.1. Chemicals

Unless stated otherwise, all chemicals were of analytical grade and purchased from Sinopharm Chemical Reagent (Shanghai, China). Acrylamide (ultra-pure grade, >99.9%) and methylcellulose (MC) were purchased from Aladdin Reagent (Shanghai, China). N, N'-methylen-bis-acrylamid (Bis), N, N, N′, N′-tetramethyl- ethylenediamine (TEMED) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Bio-Lyte pH 3–10 and 6–8 carrier ampholytes were purchased from Bio-Rad (Hercules, CA, USA). Fused silica capillary 600 μm i.d. without polymer coating was purchased from Ruipu Co. (Hebei, China). Ultra-pure water was prepared with a commercial ultrapure water system (SG, Wasseraufbereitung und Regenerierstation Gmbh, Germany).

2.2. Fabrication of array IEF device

The design of the IEF platform was outlined in Fig. 1A. The basic unit of polymethyl methacrylate (PMMA) device, briefly, had a pair of symmetrical electrode trays. The base plate of the tray was etched with twelve 1.2-mm wide channels for the assembling of the capillary array, which spaced 4 mm apart from each other. Corresponding to the channels, one of the side plates was manufactured as a comb-like structure, consisting of thirteen watertight shutters with 4 mm in width and 5 mm in height. Due to the surface tension of liquid and sufficiently small dimension, the gaps between the watertight shutters would not cause any leakage of the electrolytes. Fig. 1B shows the basic unit of the device. Capillaries of 20–30 mm long and 600 μm i.d. were used as disposable separation channel. The size of single-unit device was $45 \times 70\ mm^2$ (if 20 mm long capillary was used) or $55 \times 70\ mm^2$ (if 30 mm long). Capillary array could be assembled on a platform that contained multi-units of the PMMA device.

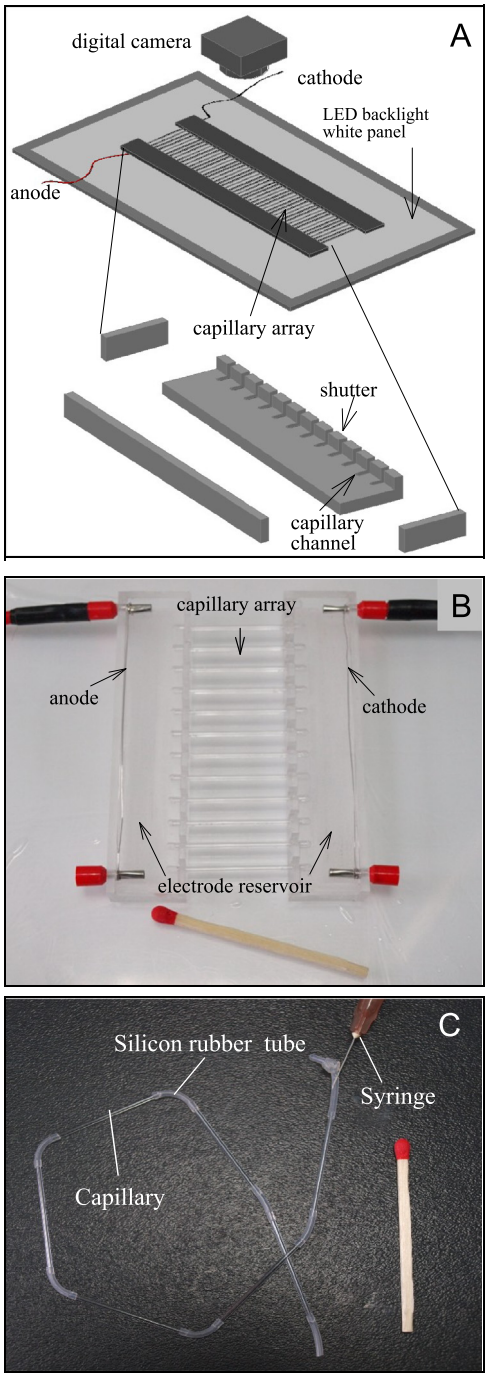

Fig. 1. (A) Schematic of the multi-unit platform for the capillary array IEF. (B) Picture of a basic-unit device with twelve capillaries. (C) The batch coating method for multiple samples.