Journal of Chromatography A, 1321 (2013) 119–126

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

A simple and highly stable free-flow electrophoresis device with thermoelectric cooling system

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ARTICLE INFO

Article history:
Received 29 July 2013
Received in revised form 1 October 2013
Accepted 18 October 2013
Available online 26 October 2013

Keywords:
Free-flow electrophoresis
Separation chamber
Joule heating
Thermoelectric cooler

ABSTRACT

Complex assembly, inconvenient operations, poor control of Joule heating and leakage of solution are still fundamental issues greatly hindering application of free-flow electrophoresis (FFE) for preparative purpose in bio-separation. To address these issues, a novel FFE device was developed based on our previous work. Firstly, a new mechanical structure was designed for compact assembly of separation chamber, fast removal of air bubble, and good anti-leakage performance. Secondly, a highly efficient thermoelectric cooling system was used for dispersing Joule heating for the first time. The systemic experiments revealed the three merits: (i) 3 min assembly without any liquid leakage, 80 times faster than pervious FFE device designed by us or commercial device (4 h); (ii) 5 s removing of air bubble in chamber, 1000-fold faster than a normal one (2 h or more) and (iii) good control of Joule heating by the cooling system. These merits endowed the device high stable thermo- and hydro-dynamic flow for long-term separation even under high electric field of 63 V/cm. Finally, the developed device was used for up to 8 h continuous separation of 5 mg/mL fuchsin acid and purification of three model proteins of phycocyanin, myoglobin and cytochrome C, demonstrating the applicability of FFE. The developed FFE device has evident significance to the studies on stem cell, cell or organelle proteomics, and protein complex as well as micro- or nano-particles.

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1. Introduction

As a stationary-phase-free separation technique, free-flow electrophoresis (FFE) has been successfully used to separate nucleic acid [1,2], protein [2–5], (stem) cell [6,7], cellular component [8–11] and enzyme [12] as well as nanoparticle [13,14]. With no anti-convection medium, adsorption material and organic extractant, FFE offers the following merits: (i) near 100% recovery [15,16]; (ii) good preservation of biological activity fitting to separation requirement of cell, organelle and protein complex as well as enzyme [15–17] and (iii) both large-scale and micro-scale biological separation [17]. These merits endow FFE great application potential in bioseparation as well as functional genomic and proteomics [2–11].

Numerous FFE apparatuses have been developed for preparative bioseparation since 1930s. In 1937, Tiselius [18] developed moving boundary electrophoresis for separation of serum proteins in free-flow solution; then Philpot [19] realized the merits of continuous FFE, such as continuous gentle separation of bioactive compounds. Hannig [20] in 1961 developed the ante-type of current FFE in which carrier buffer was run by multichannel pump. Two years later, Kolin et al. [21] designed a nice continuous-flow electrophoresis in serpentine liquid columns stabilized against thermal convection. In 1970s, Prusik et al. [22] constructed a wide FFE device for isoelectric focusing (IEF), Martin et al. [23] presented a transverse section of FFE for IEF, and Egen and Bier et al. [24–26] greatly improved FFE apparatus for recycling IEF. Meanwhile, Fawcett et al. [27] designed a short FFE for protein separation in density gradient and Rilbe [28] developed a steady-state rheoelectrolytsis. As FFE was an intricate dynamic process, the impact factors were not only plentiful but also interrelated. To optimize FFE method effectively, some mathematical models and software were developed for FFE simulation. For example, Strikler et al. [29] deduced a model for optimization of electric field, Grateful et al. [30] introduced a finite difference model for simulation of separation, Clifton et al. [31,32] constructed the models of heat and mass transfer in steady and unsteady state FFE, and Zhang et al. [33] developed the software of free-flow zone electrophoresis for optimization of

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http://dx.doi.org/10.1016/j.chroma.2013.10.058

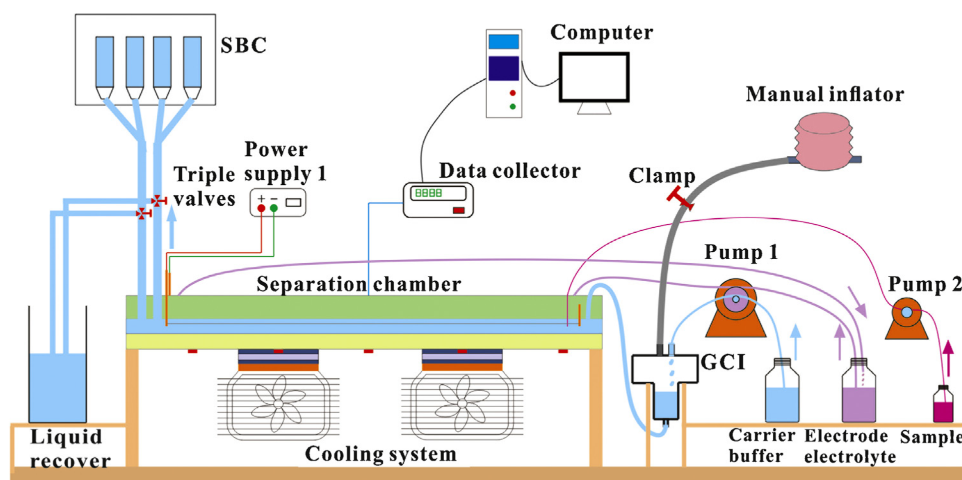

Fig. 1. Schematic diagram of overall FFE apparatus.

experimental conditions. Roman et al. [34], Krivankova et al. [35], and Herve Canut et al. [36] gave comprehensive reviews on preparative FFE in 1990s.

In many FFE devices, a multi-channel peristaltic pump had to be used for driving carrier buffer to enter the chamber, which would inevitably lead to pulse-flow of buffer. To reduce the harmful pulse-flow, we [37–39] developed a quite simple FFE device joined with gas-cushion injector (GCI), and gravity-induced self-balance collector (SBC) instead of multi-channel pump. With the device, we carried out the separation of three amino acids and sample condensation via moving reaction boundary (MRB) [40–42]. However, there were the following issues retarding application of preparative FFE. The first issue was laborious assembly of commercial FFE device (requiring half a day) due to complex steel plates and bridges used to fix chamber. The second was consuming-time bubble exhausting existing in the previous FFE [37] and commercial device (2 h or more), because the oblique angle of chamber has to been endlessly adjusted. The third was that the complex but low efficient cooling system often leading to poor control of Joule heating, regeneration of bubble in chamber, and failure of FFE separation. In addition, sometimes there was leak of solution from the chamber, and/or between electrode channels and chamber, also inducing a failure of separation and re-assembly of chamber.

Therefore, a novel preparative FFE device was developed in this paper to address these key issues. Firstly, two blocks of alloy plates were designed to form the wholly shaped upper and lower clamping plates, leading to excellent inflexibility of rectangular separation chamber without any leaking of solution. Secondly, a simple manual inflator originally used for aeration of balloon was utilized to rapidly remove any air in FFE chamber, inlets and outlets. Thirdly, a thermoelectric cooling system was designed for effectively removing Joule heating. The systemic experiments on evaluation and application of the developed device were carried out, demonstrating the merits of simple and friendly operation, and high stable performance.

2. Design of FFE device

The whole setup of newly developed FFE device was shown in Fig. 1, and the photo of experimental setup was presented in Fig. S1, including the sample separation system, and the thermoelectric cooling system.

2.1. Sample separation system

The sample separation system (Figs. 1 and 2, and Fig. S1) was constructed, based on our previous work [37–39]. The system was mainly composed of the chamber, GCI and SBC. The former was greatly improved in this paper, whereas the last two parts (GCI and SBC) were similar to the previous ones [37]. Hence, the description was focused on the new chamber.

The chamber was a “sandwich construction”, as shown in Fig. 2A. From the top to the bottom in turn were the upper plate of polymethyl methacrylate (PMMA), electroconductive membrane of anion exchange, and lower ceramic plate (Shanghai BioChemAn Biotechnology Co., Ltd, Shanghai, China). The final size of chamber was 304.1 mm × 85.4 mm × 0.7 mm. The upper plate of PMMA was designed as a frame construction to greatly improve the inflexibility and anti-leakage performance of chamber. Fig. 2B showed the fastening structure, including two alloy plates and a rubber blanket, further enhancing the inflexibility and anti-leakage ability of chamber. The center of the upper alloy plate was hollowed out for viewing of separation process. In order to equip the thermoelectric coolers, two rectangular holes were designed in the lower alloy plate.

PMMA was a kind of excellent material as shown in Table S1. Good optical transparency, easy-to-machined, high electrical insulation, and good acid–alkali resistance made it suitable for fabricating chamber. On the upper PMMA plate, 24 holes drilled near one end were inserted by high-hardness plastic tubes (o.d. 2.2 mm, i.d. 1.4 mm, height 10 mm). The 23 plastic tubes of 24 were served as inlets of carrier buffer jointed with the GCI through 23 silicon soft tubes (o.d. 2.2 mm, i.d. 1.8 mm), and the last one was used as inlet of sample injection. The position of the sample inlet could be conveniently chosen within the 24 inlets. At the other end of PMMA plate, 48 holes (divided into two rows and staggered arrangement) were drilled and also inserted by the high-hardness plastic tubes, servicing as 48 outlets for fractions in the sample collector.

Four holes were drilled at the four corners of PMMA plate to equip the electrode tips used to fix platinum wires, and upper end of the tips would be connected to a power supply (up to 1000 V, Shanghai BioChemAn Biotechnology Co., Ltd, Shanghai, China) to supply potential. Meanwhile, another four holes drilled near the four holes were also inserted by high-hardness plastic tube, and then connected the pump (BT100-1L, Shanghai BioChemAn Biotechnology Co., Ltd, Shanghai, China) to supply electrode electrolyte. Electrode electrolyte could continuously rush platinum wire to take away the oxygen, hydrogen bubbles and chemical side

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