Journal of Chromatography A, xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Journal of Chromatography A

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

The use of an inkjet injection technique in immunoassays by quantitative on-line electrophoretically mediated microanalysis

Weifei Zhang, Sifeng Mao, Jianmin Yang, Hulie Zeng, Hizuru Nakajima, Shungo Kato, Katsumi Uchiyama*

Department of Applied Chemistry, Graduate School of Urban Environmental Sciences, Tokyo Metropolitan University, Minamiohsawa, Hachioji, Tokyo 192-0397, Japan

ARTICLE INFO

Article history:
Received 18 August 2016
Received in revised form
21 November 2016
Accepted 22 November 2016
Available online xxx

Keywords:
Inkjet technology
On-line immunoassay
Electrophoretically mediated microanalysis
Capillary electrophoresis

ABSTRACT

The paper describes a new online quantitative electrophoretically mediated microanalysis (EMMA) for use in immunoassays based on the unique drop-by-drop introduction of a sample by means of an inkjet for capillary electrophoresis (CE). Plugs of a fluorescein-labeled antibody (Anti-humanIgG-DyL550) and human IgG were alternately injected into a capillary using the inkjet, followed by the merging of the plugs and the subsequent immune reaction. The antigen-antibody complex that was formed in the merged zone was then separated by CE. As a proof-of-concept, the method was used to determine human IgG. As a result, both the consumption of the reaction solution and the analysis time were significantly reduced. The method showed a wide linear range (10–2000 ng mL^{-1}, R^2 = 0.9912) of calibration and the detection limit (5 ng mL^{-1}) was substantially lower than that by for conventional methods.

1. Introduction

Capillary electrophoresis (CE) is one of the best methods for the separation of biological compounds such as DNA, protein and biomarkers. Among the several CE modes, capillary electrophoresis immunoassay (CEIA) continues to have a number of advantages over conventional immunoassays (radioimmunoassays and enzyme-linked immunosorbent assays). These advantages include low sample volume requirements, ease of automation, the ability to simultaneously detect multiple compounds, and high speed [1–4]. This flexibility offers a wide spectrum of choices for selecting different separation variants for a specific system. The introduction of electrophoretically mediated microanalysis (EMMA) was originally reported by Bao and Regnier in 1992 and remains a commonly used technique for online CE enzyme assays [5].

The EMMA methodology [6,7] couples all necessary operations into one integrated technique utilizing the different electrophoretic mobilities of analytes and analytical reagents to initiate reaction inside a capillary followed by separation of the components of the reaction mixture for final on-capillary quantification [8]. The EMMA concept has been used in studies of a variety of immunological and enzyme reaction systems. For example, Miki et al. investigated an on-column immunological reaction method using CE-SLIF in a binding assay of anti-HSA and an immunoassay of native HSA. The method provided for rapid analyses of both the binding assay and immunoassay [9]. Hai et al. developed a capillary electrophoresis-based method coupled with an enzymatic reaction inside the capillary for the screening of matrix metalloproteinase (MMP) inhibitors [10]. Avila and Whitesides have also reported on an on-column enzyme assay, in which an enzyme and its substrate are introduced into a capillary in the form of individual plugs, which are then electrophoretically mixed and react on the column [11]. Zhao et al. reported on the use of EMMA for the fast online discrimination and determination of substrate enantiomers [12]. The sensitivity was not satisfactory. Thus, developing new methodology for use in immunoassays in which very small samples are involved continues to remain a significant challenge.

Inkjet technology has been used as a research tool in various industrial fabrication processes due to its flexibility and low cost [13]. It easily permits liquid injection at the nano-/pico-liter level. Moreover, the speed and volume of ejected sample droplets can be tightly controlled using inkjet technology. This technology has generated increased interest in biochemistry, as it offers a practical and efficient method to dispense biological and material elements, including protein arrays and cell patterns [14–18]. In previous studies, we reported on the applications of inkjet technology to analytical chemistry, such as capillary electrophoresis (CE) [19,20],

* Corresponding author.
E-mail address: uchiyama-katsumi@tmu.ac.jp (K. Uchiyama).

http://dx.doi.org/10.1016/j.chroma.2016.11.041
0021-9673/

Gas chromatography (GC) [21], chemiluminescence (CL) [22], and mass spectrometry (MS) [23], Chen et al. established the sandwich CLIA system using inkjet technology by combining a multicapillary glass plate and microbeads. This method has the advantages of high sensitivity, high speed, high throughput, and automation [24].Yang et al. developed a quantitatively controlled immunoassay method that was based on inkjet technology to perform nano-liter immunoassays in a capillary microreactor [25]. Chen et al. report a novel sample introduction technique and chemical reaction strategy by drop-by-drop inkjet injection for an electrophoretically mediated microanalysis. The method results in an improved product yield for in-capillary reactions between amino acids and NBD-F [26]. Herein, based on such inkjet technology, we report on its use in the plug-by-plug EMMA analysis mode by the successive injection of pL ~ nL reagent.

In this paper, we present a highly quantitative on-line EMMA procedure for immunological analysis using inkjet injection technology for the first time. After alternately injecting an antigen and a fluorescence labeled antibody into a capillary, EMMA was carried out. The capillary acts as a micro-reactor and effectively enhanced the efficiency of the overall reaction, due to the short diffusion distance between the reactants [27]. The method permitted the antigen-antibody complex to be quantitatively determined by CE-LIF.

2. Experimental

2.1. Reagent

The anti human IgG-Fc fragment antibody (conjugateDyLight550), purified human IgG-Fc fragment, and human reference serum was purchased from Bethyl Laboratories (Tokyo, Japan). Phosphate-buffered saline (PBS) was obtained from Sigma-Aldrich Co. LLC. (Ichikawa, Japan). Boric acid was bought from Wako Pure Chemical Co. (Osaka, Japan). Extran MA01 alkaline was purchased from Merck Ltd. Japan (Tokyo, Japan). All solutions were prepared using deionized water prepared by means of a Millipore-Q system (Millipore Japan Co., Tokyo, Japan) and were filtered with a 0.45 μm membrane filter (4700, Nihon Millipore) before use.

2.2. Apparatus

The four-channel inkjet microchip was provided by Fuji Electric Co., Ltd. (Tokyo, Japan). A two-dimensional electromotive X-Y stage MMU-30X (Chuo Precision Industrial Co., Ltd. Tokyo, Japan) was used to hold the inkjet microchip and control its exact position so as to match the nozzle of the inkjet microchip and the inlet of the capillary tip. A VW-900 high-speed microscope was obtained from the Kyence Corporation, (Osaka, Japan) and was employed to observe the droplet ejected by the inkjet. A laboratory-made high-voltage power supply (0–20 kV) was used for both EMMA and the CE separation. An in-house built laser-induced fluorescence (LIF) detection system for signal detection consisted of a 532 nm of green laser (3010132, 5 mW, Laser Create Corp, Tokyo, Japan), with a filter set (U-CMAD3 Olympus, Tokyo, Japan) and a photomultiplier tube (PMT) (H6780-02, Hamamatsu Photonics, Hamamatsu, Japan) for the detection of fluorescence signal. A 75 nm hematocrit tube was utilized to contain and supply solution to inkjet microchip was from Funakoshi, Co. (Tokyo, Japan). A 50 cm long (effective length 40 cm), fused-silica capillary with an inner diameter of 100 μm and outer diameter of 375 μm was used. (GL Science, Tokyo, Japan). A chromatographic data-processing system (LAS5 Soft, Chiba, Japan) was employed to record the fluorescence signal. A HM-25G pH meter (TOADKK, Tokyo, Japan) was used to measure the pH values of the solution.

2.3. Inkjet system

The inkjet microchip was first pretreated using a previously reported method [19]. We used homemade hardware and software for the CE inkjet nano injection system [26]. The driving waveform applied to the piezoelectric device on the inkjet was controlled by a homemade driver (voltage: 0–100 V, pulse width: 0–225 μs). The exact position of the inkjet on the capillary was automatically controlled by an electromotive x-y stage via homemade software. The platform was used to evaluate the effect of injection volume by controlling the ratio of droplet numbers from different inkjet channels. (one channel was loaded with an Anti-humanIgG-DyL550 solution, and the other with Human IgG).

2.4. Off-line immunoassay

For the off-line immune assay, 1 mL mixture of 10 μg mL^{-1} Anti-humanIgG-DyL550 and 2 μg mL^{-1} Human IgG were freshly prepared with 50 mM PBS buffer (pH 6.0) as a solvent, and then incubated for 60 min at 37 °C. Immune complex was determined by the absorbance of the products at 562 nm. The product were also introduced into the capillary by inkjet with a driving voltage of 46 V and a pulse width of 20 μs and they were then separated by CE with 50 mM borate buffer (pH 8.5) as the running buffer (effective length of the capillary: 40 cm long, 100 μm i.d., LIF detection used was the same as on-line method). The electric field strength for CE analysis was 160 V/cm.

2.5. Detection of human serum

Human serum was used as the target analyte to perform a practical test. In our experiments, serum was used as a model human serum and was diluted with 50 mM PBS buffer (pH 6.0).

3. Results and discussion

3.1. Plug-by-plug immune reaction in the capillary by EMMA

Immunological reactions (antigen-antibody formation) can be achieved by the successive and alternate introduction of sample and reagent solutions, followed by zone overlapping. The effects of a pulse width of 20 μs on the volume of the ejected droplet were investigated by a gravimetric method [28], while the driving voltage of the piezo element was held 46 V constant. The results are listed in Table S1. Under the optimal ejection conditions, the volume of a single-droplet for the solution of Anti-humanIgG-DyL550 and Human IgG solutions were 216 pL, and 204 pL, respectively.

To achieve online immunoassay and to determine the amount of the immune-complex, EMMA analysis, was carried out, as shown in Fig. 1. In this procedure, fluorescence labeled Anti-human IgG-DyL550 and human IgG solutions were introduced by an inkjet and both zones were then merged, based upon the difference of electrophoretic mobilities. The reaction was then allowed to proceed. After the reaction reached completion, resulting complex and excess antibody were separated by CE.

3.2. Optimization of the injection pattern

The feasibility of present EMMA plug-by-plug injection mode was investigated for use in online immunoassays. The exact plug-by-plug overlapping was found to be a critical factor for on-line reactions. Standard samples of 10 μg mL^{-1} Anti-humanIgG-DyL550 and 2 μg mL^{-1} Human IgG were used as test samples.

In the online immune-reaction procedure, the applied voltage for achieving plug overlapping was kept constant at 3 kV. And various introduction patterns for the Anti-humanIgG-DyL550 and

Please cite this article in press as: W. Zhang, et al., The use of an inkjet injection technique in immunoassays by quantitative on-line electrophoretically mediated microanalysis, J. Chromatogr. A (2016), http://dx.doi.org/10.1016/j.chroma.2016.11.041