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Electrowetting-on-dielectric actuation of droplets with capillary electrophoretic zones for off-line mass spectrometric analysis

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ABSTRACT

Present article describes a novel technique based on digital microfluidics that allows collecting fractions of interest after electrophoretic separation and detection for further ESI-MS investigation. In this technique, a mixture is injected into a capillary electrophoresis (CE) apparatus, and microliter droplets are generated at the CE outlet at a frequency high enough to fraction each compound into several droplets, compartmentalizing the CE zones into a sequence of droplets. The droplets are transported from the CE outlet to a storage tube inlet using electrowetting-on-dielectric (EWOD) for droplet actuation. By applying a vacuum at the other end of the storage tube, the droplets form a sequence of plugs separated by air gaps. The plugs stored in the tubing are later analyzed using a standalone spectrometric device. Off-line electrospray ionization mass spectrometry (ESI-MS) was used to characterize the corresponding vitamin and was performed by pumping the segmented plugs directly into a spray emitter tip. The technique could be of interest to laboratories without access to well-equipped facilities (e.g. clean-rooms or lab robots).

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1. Introduction

Capillary electrophoresis (CE) is an alternative technique to liquid chromatography for performing separations in proteomic [1], metabolomic [2] and pharmaceutical [3] research. The ability to reliably interface CE separations with mass spectral (MS) analysis has therefore become increasingly important [4]. However, the nature of CE gives rise to some particular challenges with regard to on-line MS detection. The principal method of on-line CE-MS interfacing is electrospray ionization (ESI). Both the CE and ESI processes require stable electrical contact of the solution with an electrode at the capillary outlet without interrupting the electro-osmotic flow from the CE separation. In addition, the low volumetric flow rates used in CE impose restrictions on the geometry of the tip, if a stable electrospray is to be maintained. The compatibility of the background electrolyte with the electrospray process and on the resulting mass spectra must also be considered.

Off-line analysis of CE fractions is somewhat simpler. Although this is not a new approach, the problem is still relevant and novel solutions are actively being sought. The use of capillary electrophoresis to collect fractions was first demonstrated by Hjerten and Zhu in 1985 on nucleosides, pH markers and IEF ampholytes [5]. Therefore, off-line coupling systems, which allowed automated protein spotting, were introduced [6]. Zamfir et al. suggested the use of off-line high-performance capillary electrophoresis in combination with nanospray ESI Q-TOF [7,8]. In the first microchip presented by Effenhauser et al., the separation channel of the CE fractionator had two exits: one was used for collection, while the other served as a waste channel. By alternating the applied potentials at predetermined times and closing an electric circuit, the fractions could be drawn through either channel [9]. A microfabricated device capable of selecting and collecting multiple components from a mixture separated by CE was recently described by Zalewski et al. [10]. In the work by Barbula et al., effluent from capillary columns is deposited on a rotating Teflon disk that is covered with paper. As the surface rotates, the temporal separation of the eluting analytes (i.e., the electropherogram) is spatially encoded on the surface [11]. Desorption electrospray ionization is then used for ionization and MS analysis of the deposited sample.

However, off-line analysis and characterization of samples separated by CE has been problematic even with conventional approaches to fraction collection [12–17]. Droplet-based microfluidics could provide a novel means for using MS to analyze the separated compounds. Droplets or plugs within multiphase microfluidic systems are rapidly attracting interest as a way to manipulate samples and chemical reactions on the scale of femtoliters to microliters. Developments in droplet manipulation such as passive and active fusion, splitting and sorting have enabled more complicated and integrated experiments [18,19]. There has been substantial recent interest in integrating CE and LC separations

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with droplet-based microfluidics. Edgar was the first to identify a concept based on the use of droplets to compartmentalize the separated CE bands [20]. An approach by Niu et al. efficiently integrates two separation dimensions: the first dimension – capillary HPLC separation – generates, segments and recombines droplets in a continuous flow, followed by the second dimension – CE separation [21]. Doshi et al. described the formation of heterogeneous libraries of droplets using fractionation of a sample into droplets by means of a C18 cartridge [22,23]. By interfacing nanoflow ultra-performance liquid chromatography with droplet-based microfluidics, Theberge et al. developed a method to produce libraries of picoliter droplets of chromatographic zones [24].

Challenges remain with chemical analysis of droplets in general and MS analysis in particular. Helmja et al. demonstrated droplet-based fraction collection in capillary electrophoresis for various stand-alone mass spectrometers [25]. Kennedy's group used oil segmented flow fraction collection from capillary LC and off-line ESI-MS [26,27]. Collections of nanoliter fractions are stored in tubing as plugs that can later be used to manipulate the samples. Off-line ESI-MS was used to characterize samples by pumping the segmented plugs directly into a nanospray emitter tip.

In most of these systems, droplets form spontaneously when a laminar stream of aqueous effluent is injected into an immiscible carrier fluid, either at a T-junction or in a flow-focusing geometry [28]. Oil must be depleted prior to MS analysis, and this requires a special step. Kennedy et al. [26] siphoned the oil away from the ESI tip. Electrowetting on dielectric (EWOD) can be used to actuate droplets instead of using a carrier liquid. According to [29,30] electrowetting was firstly discovered by French physicist Gabriel Lippman in 1875. Further, this technique has been developed to electrowetting on dielectric [31–35] and found many applications in digital microfluidics, where discrete droplets of conductive aqueous solutions could be manipulated by electrostatic forces on an array of electrodes coated with an insulating dielectric. Recent developments in this field [36,37], include proteomics analyses [38–40], immunoassays [41–44], and microfluidic platforms capable of all the steps required for mammalian cell culture: seeding, growing, detaching, and re-seeding the cells on a fresh surface [45].

However, despite much enthusiasm for this approach, access to digital microfluidics devices continues to be a barrier for researchers without access to well-equipped facilities (mainly, clean-rooms). This has spurred research into methods of fabrication that are robust and easy to implement [46], including prototyping on copper laminates using commercial printers [47,48]. Here, we report an interface for off-line CE-MS coupling which is based on a robust EWOD platform. It is used to compartmentalize CE zones into droplets, which are stored as plugs in a Teflon tube for ESI-MS analysis for further MS analysis. In our system, CE zones that emerge at the capillary outlet are dissolved in microlitre droplets of pure (MS compatible) solvent generated by an external droplet generator and transported away from the outlet of the separation capillary by EWOD. This is a novel approach in the field of fraction collection. As above, droplets are transported to a storage tube for ESI-MS analysis. The concept has been proven by an ESI-MS analysis of vitamins in a standard solution as well as in commercial formulations.

2. Materials and methods

2.1. Chemicals

Thiamin hydrochloride (vitamin B1), thiamin monophosphate (phosphorylated vitamin B1), and pyridoxine (vitamin B6) and the reagents (acetic acid, methanol, acetonitrile, and sodium hydroxide) were purchased from Sigma–Aldrich (Germany). The purity of the chemicals was 98% or higher. Deionized water (pH 7.0) purified with a MilliQ system (Millipore Corporation, Bedford, USA) was used for the preparation of the standard solutions.

Three commercial vitamin solutions purchased from a local pharmacy were tested. The first sample was a Milgamma® N injection solution (a medication prescribed for diabetics) with concentrations of B1 (148 mmol/L), B6 (243 mmol/L) and lesser amounts of interfering compounds such as lidocaine (43 mmol/L), cyanocobalamin, and benzyl alcohol. The second sample was Apovit "Lion Boy" (a potable liquid food supplement manufactured by Nycomed SEFA AS Estonia) that contains B1 (300 μmol/L), B6 (710 μmol/L), as well as various other components. The third sample was "Doppelherz" (a liquid food supplement comprised of an enriched mixture of herbal extracts, from Queisser Pharma) containing B1 (189 μmol/L), B6 (296 μmol/L).

Epoxy single-sided copper clad laminate (copper layer 17.5 μm; Elfa Elektroonika AS) was used as the substrate for fabricating the digital microfluidics actuator (DMFA). Other fabrication materials included silicone oil (PRF silicon oil, Taerosol Oy, Finland), food wrap with an estimated thickness of 10 μm (Lindner Haushaltsprodukte GmbH, D-51149, Köln), poly[4,5-difluoro-2,2-bis(trifluoromethyl)-1,3-dioxole-co-tetrafluoroethylene] dioxole 87 mol% (Teflon AF, DuPont), and Fluorinert FC-75. Gold paint (Glanzgold NF 12% solution of gold, Schjerning, Denmark) and silver paint (Silver Conductive Paint, Electrolube, England) were used to treat the outlet of the separation capillary as well as the grounded electrodes.

2.2. Instrumentation

In present study the authors suggest a new application of the previously described in [53] CE-DMF plate instrumentation, where DMF platform has been combined with CE instrumentation for repeated computer controlled sampling from a droplet (using EWOD for droplet manipulation on the surface of the DMF platform) and electrophoretic analysis with C^4D. On contrary to earlier research here we use DMF platform not as a sampler but as a collector and transporter of CE fractions either into the storage tube (if they contain analyte) or to waste (if empty). Selection was made according to the C^4D detector signal. Air plugs instead of a carrier liquid were used for encapsulation of the selected fractions. Air segmentation solves several problems encountered in on-line connection of storage tube with ESI-MS. First, there is no risk for the MS source to be contaminated since the zones are separated only by air. Second, no complicated instrumentation modifications are needed for purification of fractions from the carrier liquid.

2.3. Capillary electrophoresis

The CE instrument with a capacitively coupled contactless conductivity detector (C^4D) and the DMFA for the droplets were purpose-built instruments. A schematic diagram of the instrument is provided in Fig. 1A. The CE system requires power supply (Spellmann, Hauppauge, NY) capable of delivering voltages up to +25 kV. The C^4D was constructed according to the ideas outlined by da Silva et al. [49] and Zemann et al. [50] and has already been used for various applications [51,52]. It operates at 60 V peak-to-peak sine wave oscillating in a frequency 200 kHz. The instrument is controlled and the signal obtained via a USB connection with software developed in-house. The separation was effected in a 70 cm (effective length 35 cm) fused-silica capillary (Polymicro Technologies, Phoenix, AZ), with an outer diameter of 150 μm and an inner diameter of 75 μm. The surface of outlet (ground) end of the separation capillary was covered first by gold paint and heated at 600 °C for 2 min. After cooling the outlet end of capillary was painted by silver paint, leaving the end of the capillary covered only with gold to enable

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