Journal of Chromatography A, 1466 (2016) 173–179

Contents lists available at ScienceDirect

Journal of Chromatography A

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

Interface-free capillary electrophoresis-mass spectrometry system with nanospray ionization—Analysis of dexrazoxane in blood plasma

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ARTICLE INFO

Article history:
Received 23 June 2016
Received in revised form 16 August 2016
Accepted 18 August 2016
Available online 23 August 2016

Keywords:
Capillary electrophoresis
Mass spectrometry
Nanospray
Hydrophobic coating
Interface
Dexrazoxane

ABSTRACT

The newly developed interface-free capillary electrophoresis-nanospray/mass spectrometry system (CE-nESI/MS) was applied for rapid analysis of the cardioprotective drug dexrazoxane and its hydrolysed form ADR-925 in deproteinized blood plasma samples. The aim of this study was to test the simplest possible CE-nESI/MS instrumentation for analyses of real samples. This interface-free system, utilizing single piece of a narrow bore capillary as both the electrophoretic separation column and the nanospray emitter, was operated at a flow rate of 30 nL/min. Excellent electrophoretic separation and sensitive nanospray ionization was achieved with the use of only one high voltage power supply. In addition, hydrophobic external coating was developed and tested for additional stability of the nanospray ionization. To our knowledge this is the first study devoted to the analysis of dexrazoxane and ADR-925 by capillary electrophoresis-mass spectrometry.

1. Introduction

Capillary electrophoresis (CE) can provide efficient separation of ionic species according to their electrophoretic mobility. The separation is usually performed in fused silica capillaries with inner diameters of 50–150 μm, where narrower tubes result in lower Joule heating and allow the use of higher electric field strength and/or background electrolyte (BGE) with higher conductivity [1–4]. Another important aspect of separations in narrow bore channels is a substantial decrease of the sample consumption making CE useful especially if limited sample is available, e.g., in single cell lysates [5]. The diameter reduction of the separation capillary results also in the reduction of flow rate necessary for transporting the separated zones into the electrospray. At the very low flow rates (below 100 nL/min) the ionization efficiency increases together with the robustness towards the solvent composition and ion suppression effects decrease [6,7]. This was recently demonstrated by Moini and Rollman running CE-nESI/MS analysis at a flow rate of 10 nL/min, allowing high sensitivity analyses even with nonvolatile chiral selector (sodium salts of cyclodextrin) in the BGE [8]. Similarly, Mayboroda et al. investigated signal of multiply phophorylated peptides within 6.6–100 nL/min during MS infusion. An increase in ionization efficiency of phophorylated peptides was observed at decreased flow rates. Moreover, nanospray operated at ≤20 nL/min provided nearly equimolar response of the tested analytes as a result of significantly reduced ion suppression effect [9].

While it is clear that nanospray brings the best ionization performance, there are different ways of coupling it with the capillary electrophoresis. Two basic groups can be distinguished based on the interface construction with respect to the electric current connection at the electrospray end of the separation capillary. In the first group of the coaxial sheath liquid arrangement [10], an additional conductive liquid (spray liquid) is added for the transport of the separated ions into the nanospray tip. Similar arrangement called a liquid junction [11–13] allows the use of additional liquid with or without any additional flow (pressure or electroosmotic). The electric currents circuits for both the electrophoretic separation and electrospray ionization can be closed via an electrode in contact with the spray liquid. This design has been recently optimized for high sensitivity separations [14–16]. The same principle can be also used in microfabricated devices for mass spectrometry [17,18]. The second group uses a conductive component e.g. attached tip, tip coating or porous glass membrane for electrical connection [19]. Generally, the interfaces implementing a spray liquid provide wider

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http://dx.doi.org/10.1016/j.chroma.2016.08.042
0021-9673/

operation range with respect to the capillary size and buffer composition, whereas sheathless designs may reach better sensitivity [20,21].

Recently, we have proposed a type of interface-free design [22] where the electrophoretic separation is performed in a long narrow bore capillary (ID ≤15 μm) serving also as the nanospray tip. In this design the electrospray current was connected at the injection end of the separation capillary and the resistance of the narrow separation channel created sufficiently high potential drop for the CE separation. Thus only one high voltage power supply was needed with no additional electric connection at the electrospray capillary end making the instrument extremely simple.

It should be mentioned, that the shape of the electrospray tip strongly influences the ionization performance. If the tip is blunt, asymmetric or its surface is rough (cause of higher wettability), the base of the Taylor cone tends to spread resulting in the loss of ionization efficiency, signal stability and resolution of separated zones [23–25]. The volume of Taylor cone can be held at minimum level if the emitter tip is sharp and smooth [26]. Significant improvement can also be achieved with emitters from hydrophobic materials or with hydrophobic surface coating [27,28].

Here we present the interface-free system for CE-nESI/MS with hydrophobically coated nanospray tip for repeatable and sensitive simultaneous analysis of dexrazoxane and its metabolite ADR-925 in blood plasma.

2. Material and methods

2.1. Chemicals

Dexrazoxane, ammonium formate, formic acid and all the solvents were purchased from Sigma Aldrich, (MO, USA). ADR-925 was synthesized by procedure described previously [29].

2.2. Sample preparation

Stock solution of dexrazoxane was dissolved in methanol in 1 mg/mL concentration. ADR-925 was prepared in concentration of 0.1 mg/mL in 50% methanol (v/v).

The rabbit plasma sample was deproteinized by mixing with six volumes of acetone and vortexed approximately for 30 s. The mixture was centrifuged at 14 000 rpm for 10 min. To concentrate the analytes and to avoid the difficulties resulting from high acetone percentage, 100 μL of supernatant was evaporated in the vacuum evaporator (20 min) and dissolved in 10 or 50 μL deionized water.

Samples for in vivo study were obtained from a rabbit treated by 60 mg/kg dose of dexrazoxane. Blood was collected after 10, 20, 60, 120 and 180 min post dose and processed to plasma. The dose, route of administration and experimental setting of the in vivo experiment was used as described previously in the study of dexrazoxane-afforded cardioprotection [30]. Each sample was divided into three aliquots to quantify dexrazoxane by the method of standard addition. Dexrazoxane was added to the sample to reach its final concentrations of 0, 25 and 50 μg/mL. The rest of the sample treatment was the same as described above for the spiked plasma samples. All the plasma samples were kindly provided by Dr. Sterba (Faculty of Medicine in Hradec Kralove, Czech Republic).

2.3. Fabrication of nanospray tip

The nanospray tip was fabricated at the end of a 60 cm long fused silica capillary with 15 μm ID (Polymicro Technologies, AZ, USA) by grinding. The inner capillary diameter remains unchanged during the grinding process. The tip angle was set to 5° using the grinding device assembled from 3D printed components [26]. To support the electrospray Taylor cone stability the tip was treated by a hydrophobic coating prepared from a mixture of the Teflon® AF in the form of 60% water dispersion (DuPont CZ, Prague, Czech Republic) and transparent UV top coat nail polish (Dermacol, Brno, Czech Republic) in volume ratio 3:1. The tip was horizontally dip-coated into the dispersion for 5 s. To prevent tip clogging the capillary was flushed by nitrogen at 4 atm. The coating was dried at laboratory temperature in vertical position for 5 min, treated by heat gun at 370 °C for 3 min and exposed to UV lamp for 30 s to harden the coating.

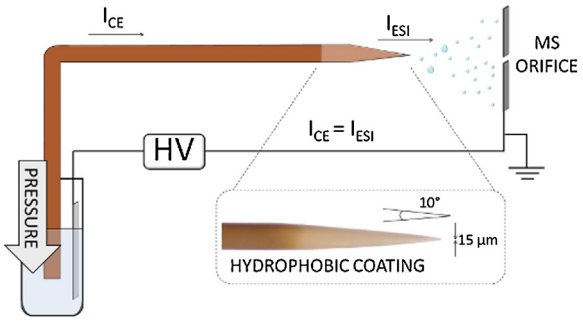

Fig. 1. The scheme of the CE-nESI/MS instrumentation.

2.4. Characterization of tip surface

The surface of fabricated tips was inspected under scanning electron microscopy (MIRA3, Tescan, Brno, Czech Republic). The hydrophobicity of the coating was investigated by contact angle measurement on See System (Department of Physical Electronics, Masaryk University, Brno, Czech Republic).

2.5. CE-nESI/MS

Electrophoretic separations were performed in the sheathless and electrodeless arrangement as described earlier [22]. Briefly, the CE separation was conducted in a bare fused silica capillary (60 cm, 15 μm ID) with the nanospray tip at one end. The tip was positioned in front of the MS sampling orifice without any additional electrical connection. The opposite (injection) capillary end was placed in a polypropylene vial inside a nitrogen pressurized chamber allowing control of the flow rate in the capillary as well as sample injection. Using a 0.5 atm pressure for 10 s, approximately 1 nL of the sample was loaded. The CE separation current was delivered by SL 10 W–300 W power supply (Spellman High Voltage Electronics, United Kingdom) via a Pt electrode inserted into the polypropylene vial. Aqueous solution of 1.5% formic acid (v/v; pH = 1.9) was used as the BGE. The analysis was divided into two steps. In the first step, after the sample injection, high voltage (30 kV) was applied under no flow conditions. During this step, the separation current (3.5 μA) caused fast migration and separation of the sample ions and also resulted in the formation of corona discharge at the capillary tip, unsuitable for mass spectrometry analysis. After 4 min, the voltage was adjusted to the nanospray friendly level of 5 kV (the ESI current was measured in an independent experiment as ~0.1 μA) and the BGE flow of 30 nL/min inside the separation capillary was initiated by pressurizing the electrode reservoir. Thus, the sample zones, already separated in the first step, were transported to the nanospray tip, electrosprayed, and detected by MS under optimum nanospray conditions. The scheme of used CE-nESI/MS instrumentation is in Fig. 1.

All MS experiments were conducted on the Velos Pro Dual-Pressure Linear Ion Trap Mass Spectrometer (Thermo Fisher Scientific, Germany) in the positive ionization mode. The nanospray