Journal of Chromatography A, 1488 (2017) 134–139

Contents lists available at ScienceDirect

Journal of Chromatography A

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

One-step selective electrokinetic removal of inorganic anions from small volumes and its application as sample clean-up for mass spectrometric techniques

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ARTICLE INFO

Article history:
Received 3 November 2016
Received in revised form 24 January 2017
Accepted 26 January 2017
Available online 27 January 2017

Keywords:
Sample clean-up
Inorganic anions
Mass spectrometry
Electrophoresis

ABSTRACT

The presence of inorganic anions in a sample interferes with mass spectrometric (MS) analysis. Here, a simple method to remove these ions from a liquid sample in one-step is described. The inorganic anions present in a 50 μL sample were extracted into a low pH solution inside a 200 μm i.d. × 33 cm long capillary by the use of an electric field. The selective removal of unwanted anions and retention of target analytes was accomplished by control of the apparent electrophoretic velocities of anions and analytes at a boundary that separated the sample and extraction solution. No physical barrier (e.g., membrane) was required and with the boundary situated at the tip of the capillary, efficient removal of inorganic anions (e.g., >80% removal) and good recovery of target analytes (e.g., >80% recovery) were achieved. The time required for removal of the inorganic anions was found to depend on their initial concentrations. The removal process was investigated using different concentrations of bromide and nitrate (as potassium salts) and negatively chargeable drugs as target analytes. This micro-sample clean-up technique used no organic solvents and little consumables and was studied to the determination of 0.6 μg/L arsenic and 8.3 μg/L vanadium in 500 mg/L sodium chloride using inductively coupled plasma MS and 50 μM angiotensin I in 1000 mg/L sodium chloride using electrospray ionisation MS. Micro-sample clean-up was performed for 45 min at 3 kV in both demonstrations. The calculated recoveries for the metals at trace levels were 110–130%, and for the peptide was 103.8%.

1. Introduction

The mass spectrometric (MS) analysis of environmental, food, biological, and other samples can often be problematic when inorganic ions are present in the samples [1–4]. In inductively coupled-plasma-MS (ICP-MS) with standard sample introduction into an argon (Ar) plasma, high chloride matrices can result in the formation of polyatomic molecular interferences, especially for arsenic ($^{75}As^{+1}$from $^{40}Ar^{35}Cl^{+}$) and vanadium ($^{51}V^{+1}$ from $^{35}Cl^{16}0^{+}$). In low resolution (high sensitivity) mode ICP-MS, chloride levels of around 1 g/L may lead to signals equivalent to 0.5 μg/L of arsenic and 26 μg/L of vanadium. Thus, the presence of chloride prevents the reliable quantitation of these metals at the μg/L levels. In ESI–MS, there is a loss of ionization efficiency due to the presence of high concentrations of inorganic salts and this is commonly known as matrix effects that cause signal suppression or enhancement [5]. The accumulation of salts over continuous use of equipment also negatively affects the performance of ESI–MS instruments [6,7].

For both ICP-MS and ESI–MS, the most commonly applied sample preparation methods for removal of inorganic ions (desalting) have been solid-phase extraction [8,9], liquid-liquid extraction [10,11], electrophoresis [12–14], chromatography [15–18], and dialysis [19,20]. These methods are typically labour-intensive, time consuming, and can result in loss of analytes when applied to small volume samples. In ICP-MS, spectral interferences can be minimized using the high resolution mode, but results in an increase in the limit of detection. The addition of interference reduction technologies in ICP-MS instrumentation, such as collision and reaction cells, has also been developed to solve this issue [21–24].

In ESI–MS, innovative microscale devices mainly integrated online have been reported. For example, Dasgupta and co-workers developed a three-compartment flow-through device that allowed the salt ions to be removed from the central channel through two ion-exchange membranes (anion and cation) under the influence of an electric field [25]. Federov and co-workers developed a microfabricated device that utilized the difference in diffusivities of ions and analytes through a high counter-flow rate of sample solu-

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http://dx.doi.org/10.1016/j.chroma.2017.01.073
0021-9673/

tion and salt-free buffer, which were separated with an alumina membrane [26]. More recently, Zhang and co-workers reported membrane ESI (capable of reducing matrix effects from salts) that was inspired from paper spray ionization [27].

The selective electrokinetic removal of cationic species from small sample volumes under field-enhanced conditions [28] in capillary electrophoresis (CE) has been reported previously [29]. The efficient removal of cationic species was obtained in samples with very low salt content. In the present study, a simple electrokinetic micro-sample clean-up technique to selectively remove inorganic anions from liquid samples with high salt content is described. This new membrane-free technique uses an acidic extraction solution (ES) inside a 200 μm internal diameter (i.d.) capillary. The ends of the 33 cm long capillary were dipped into a 50 μL sample and ES. An electric field was used to selectively separate the inorganic anions from the target ionizable analytes in the sample by transfer of the inorganic anions into the ES inside the capillary. The separation was accomplished by control of the apparent electrophoretic velocities of analytes and inorganic anions at the tip of the capillary which was in contact with the sample. Significant removal of inorganic anions and high recovery of the target analyte species which remained in the sample solution were achieved. This environmentally friendly analytical sample preparation technique was demonstrated for the analysis of arsenic and vanadium by ICP-MS, and also for a peptide (angiotensin I) by ESI–MS in the presence of high concentrations of sodium chloride (NaCl) in the sample. Inorganic cations such as sodium ions, however, are retained in the sample and formed adducts with the analytes in ESI–MS. The adduction of alkali metal ions to analytes is a common occurrence in ESI–MS [30,31]. The adducts formed often have higher sensitivities than the protonated or deprotonated molecules. The adducts can be used for quantitation [32,33]. Algorithms have been developed to accurately determine the molecule represented by molecular ion adducts in positive and negative mode in ESI conditions [34].

2. Experimental section

2.1. Reagents and stock solutions

Acetic acid (>99.8%), phosphoric acid (85 wt.%), acetonitrile (ACN, HPLC grade), sodium dodecyl sulfate (SDS, >99.0%), sodium hydroxide (NaOH, >95%), sodium chromate (Na_2CrO_4, 98%), hexadimethrine bromide (HDMB, >94%), potassium bromide (KBr, >99%), potassium nitrate (KNO_3, >99%), NaCl (>99.5%), triethanolamine (TEA, >99.0%), methanol (MeOH, HPLC grade), and angiotensin I human acetate salt hydrate (≥90.0%) were obtained from Sigma-Aldrich (St. Louis. MA). Rosuvastatin (pKa 4.0, MW 481 g/mol), furosemide (pKa 3.8, MW 330 g/mol), indapamide (pKa 8.8, MW 365 g/mol) acidic drugs (>99%) were purchased from Sigma-Aldrich or Fluka (Buchs, Switzerland). Purified water was from a Milli-Q system (Millipore, Bedford, MA, USA). Stock solutions of the inorganic anions were 10 g/L of KBr, KNO_3, and NaCl in purified water. Stock solutions of the target compounds were 1 mg/mL of each drug and 100 μM angiotensin I in purified water. Arsenic and vanadium atomic absorption standard solutions (1000 mg/L) were obtained from Sigma-Aldrich. ES was 5 M acetic acid at pH 2.5. The selection of this ES is detailed in the Supporting Info. Experimental Section. All solutions were filtered through 0.45-μm filters.

2.2. General procedure for removal of inorganic anions

The removal of inorganic anions by micro-sample clean-up was performed using a commercial CE instrument from Agilent Technologies (3D-CE) (Waldbronn, Germany). The CE instrument, which was readily available in our laboratory, was used to conveniently establish the fundamentals of the method. The extraction capillary was fused-silica from Polymicro (Phoenix, AZ) with 360 μm o.d. A wide 200 μm i.d. was used since the amount of ions electrokinetically extracted into the capillary was proportional to the capillary i.d. According to Chien [35], the total amount of ions *i* injected into the capillary in electrokinetic injection after a specified time (*t*) is directly proportional to the cross-sectional area *A* of the capillary as shown in the following equation:

$$N_i = \int_0^t AC_{is}\left(\nu_{ei}t + \nu_b(t)\right)dt$$

Where:

N_i = total amount of ion species
C_{is} = concentration of ion species *i* in the sample reservior
ν_{ei} = electrophoretic mobility of the ion *i*
ν_b = electroosmotic velocity

A study of i.d. 25, 50, 100, 150, 200 μm capillaries was performed. The biggest i.d.(200 μm) showed the highest percentage removal of inorganic ions at a given time. The shortest possible total length of 33 cm for accommodation of the capillary into the capillary cartridge was employed.

New capillaries were flushed at 1 bar with 0.2 M NaOH for 5 min, purified water for 2 min, and ES for 4 min. Pre-conditioning of the capillary before each sample treatment was carried out by flushing at 1 bar with 0.2 M NaOH for 0.5 min, purified water for 0.25 min, and ES for 0.25 min. A 50 μL sample solution was placed in the cathodic vial while the ES was placed in the anodic vial. Voltage at 3 kV with the cathode at the sample vial was applied while maintaining capillary temperature at 20 °C. Higher voltages led to voltage drops and excessive bubble formation at the sample vial, especially for high salt content samples. At the end of each working day, the electrodes were washed and stored in purified water to remove salt residues.

The Supporting Information (Supp. Info.) Experimental Section contains the information regarding the following: general CE procedure; capillary zone electrophoresis (CZE) with direct UV detection of bromide and nitrate; CZE with indirect UV detection of chloride; micellar electrokinetic chromatography (MEKC) with UV detection of drugs; direct infusion ESI–MS of angiotensin; and ICP-MS of arsenic and vanadium.

3. Results and discussion

3.1. Selective electrokinetic removal of inorganic anions through a purely aqueous boundary

Fig. 1 shows the mechanism for the selective removal of inorganic anions from a small volume sample solution (i.e., 50 μL), with the analytes being retained in the sample. (A) is the starting situation. A 200 μm capillary (33 cm) with acidic ES was dipped into the sample. The other end of the capillary was dipped in a vial with the ES. A purely aqueous boundary between the sample solution and the ES was established at the tip of the capillary that was in contact with the sample. A voltage with the cathode at the sample solution was applied as shown in (B). The resultant v_{eof} that was directed towards the sample solution was slow because of the low pH of the ES. The negatively and positively charged species in the sample migrated to the anode and cathode, respectively.

In (B), the apparent velocities (v_{app}) of inorganic anions was towards the capillary, resulting in removal of inorganic anions from the sample, whereas v_{app} for the analytes was towards the sample solution, leading to retention of analytes in the sample. The ν_{app} was the sum of the electrophoretic velocity of the compound (ν_a) and velocity of the EOF (ν_{eof}), with ν_a being equal to the product of