Analytica Chimica Acta 998 (2018) 67–74

Contents lists available at ScienceDirect

Analytica Chimica Acta

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

Methodology of analysis of very weak acids by isotachophoresis with electrospray-ionization mass-spectrometric detection: Anionic electrolyte systems for the medium-alkaline pH range

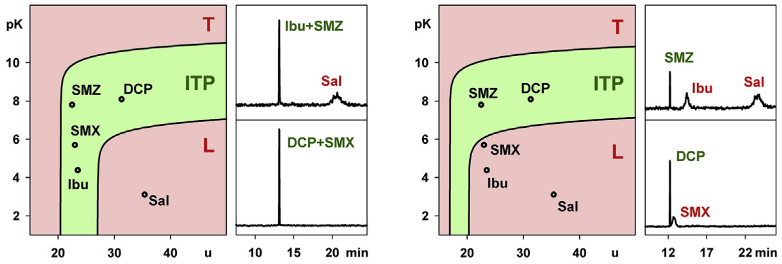
Zdena Malá, Petr Gebauer*

Institute of Analytical Chemistry of the Czech Academy of Sciences, v.v.i., Veveří 97, CZ-602 00 Brno, Czech Republic

HIGHLIGHTS

- First application of ITP-ESI-MS in the neutral-alkaline pH range.
- Flexible system setup using moving-boundary ITP.
- OH^- used as essential terminating component.
- Sensitive and selective analyses of weak acidic analytes.
- Analysis of sulfonamides in waters.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 8 August 2017
Received in revised form
29 September 2017
Accepted 2 October 2017
Available online 17 October 2017

Keywords:
Capillary isotachophoresis
ESI-MS detection
Medium-alkaline pH
Sulfonamides
Chlorophenols

ABSTRACT

This work describes for the first time a functional electrolyte system setup for anionic isotachophoresis (ITP) with electrospray-ionization mass-spectrometric (ESI-MS) detection in the neutral to medium-alkaline pH range. So far no application was published on the analysis of very weak acids by anionic ITP-MS although there is a broad spectrum of potential analytes with pK_a values in the range 5–10, where application of this technique promises interesting gains in both sensitivity and specificity. The problem so far was the lack of anionic ESI-compatible ITP systems in the mentioned pH range as all typical volatile anionic system components are fully ionized at neutral and alkaline pH and thus too fast to suit as terminators. We propose an original solution of the problem based on the combination of two ITP methods: (i) use of the hydroxyl ion as a natural and ESI-compatible terminator, and (ii) use of configurations based on moving-boundary ITP. The former method ensures effective stacking of analytes by an alkaline terminator of sufficiently low mobility and the latter offers increased flexibility for tuning of the separation window and selectivity according to actual needs. A theoretical description of the proposed model is presented and applied to the design of very simple functional electrolyte configurations. The properties of example systems are demonstrated by both computer simulation and experiments with a group of model analytes. Potential effects of carbon dioxide present in the solutions are demonstrated for particular systems. Experimental results confirm that the proposed methodology is well capable of performing sensitive and selective ITP-MS analyses of very weak acidic analytes (e.g. sulfonamides or chlorophenols).

1. Introduction

One of the crucial factors of a successful separation in capillary

---

* Corresponding author.
E-mail address: gebauer@iach.cz (P. Gebauer).

https://doi.org/10.1016/j.aca.2017.10.013
0003-2670/© 2017 Elsevier B.V. All rights reserved.

electrophoresis (CE) is the proper selection of electrolyte systems. It has been shown many times that the architecture of the wet part of the separation system may improve the sensitivity and/or selectivity of a separation better and much easier than laborious modifications of the hardware parts such as channel or detector. This fully applies also to CE with mass-spectrometric (MS) detection where suitable electrolyte setups are commonly used for transient sample stacking and sometimes also permanent stacking or focusing were successfully applied. Permanent stacking is based on isotachophoresis (ITP) where trace analytes are concentrated at a self-sharpening moving boundary and transported to the detector in form of steady-state zones. Despite the high potential of ITP-MS to raise sensitivity by several orders of magnitude, the number of so far published applications remains limited since 1989 when the ITP-MS technique was reported for the first time [1,2]. Almost all published applications are based on cationic ITP, i.e., analysis of cationogenic analytes (phosphonium ions, peptides, proteins, vitamins and pesticides) [1–7]. The first anionic ITP-MS was described in 1995 for the analysis of monophosphate nucleosides and similar compounds [8–10]; reversed polarity operation, electroosmotic-flow-assisted transport and MS detection in the positive-ion ESI mode were applied here. The first anionic ITP-MS in the negative-ion ESI mode was presented by our group in 2013 [11] for the analysis of pharmaceuticals in waters. In combination with off-line solid phase extraction, the sensitivity of this method with limits of detection below 2 pM is comparable to HPLC-MS [12].

Possible reasons of the limited interest in ITP-MS may be the restricted number of available electrolyte components compatible with electrospray-ionization (ESI) and also the fact that in ITP typically several analyte zones comigrate in one stack which does not allow using this technique for the analysis of samples with a high number of components. Our efforts to overcome these limitations resulted in the proposal of various strategies of electrolyte architecture [11,13,14]. Of them, the implementation of moving-boundary ITP to CE-MS offers increased flexibility: multiple coions present in the leading and/or terminating zones allow effective control of the stacking window and system selectivity by simply changing their concentration ratio(s). We have applied this methodology successfully both to cationic [7] and anionic ITP [13] in acidic systems. The spectrum of ESI-compatible anionic coions is limited to several substances like formic, acetic, propionic or lactic acids. The all have relatively high ionic mobilities (between approx. 37 and 56 × 10^{-9} $m^2V^{-1}s^{-1}$) and dissociation constants in the acidic range (between 3.75 and 4.87). They allow creation of functional acidic ITP systems due to partial dissociation that decreases their effective mobilities sufficiently to allow termination of analytes ionized in acidic medium.

In neutral and alkaline medium, it becomes a serious problem to set up an applicable anionic ESI-compatible ITP system because the above mentioned acids are fully ionized and too fast to serve as terminating zone components. Moreover, the potential disturbing effects of carbon dioxide on the anionic ITP boundary may bring additional complications in this pH range. Recently we have combined electrophoretic focusing on inverse electromigration gradient with ESI-MS detection and successfully applied to sensitive analysis of weakly acidic analytes [15]. To our knowledge, anionic ITP with ESI/MS detection in neutral-alkaline region has not been published so far. On the other hand, there is a large spectrum of anionic analytes that are very weak acids with pK_a values in the range between 5 and 10, where application of ITP-MS to their analysis is a challenge promising interesting gains in both sensitivity and specificity. This work is aimed at exploring the options of setting up functional ESI-compatible ITP systems in the neutral to medium-alkaline pH range. Theoretical considerations combining the concepts of OH^- used as terminator and moving-boundary ITP allow simple assessment of the parameters of a system and particularly of its stacking window. The properties of proposed systems and their applicability are demonstrated both on model substances and on the analysis of water samples.

2. Materials and methods

2.1. Chemicals

All chemicals, sulfamethazine (SMZ), sulfamethoxazole (SMX), 2,4-dichlorophenol (DCP), salicylic acid (Sal), ibuprofen (Ibu), acetic acid (all from Fluka, Buchs, Switzerland), ethanolamine (ETA), triethylamine (TEA) (both from Sigma, St. Louis, MO, USA) and ammonium hydroxide (Lachema, Brno, Czech Republic) were of analytical reagent grade and methanol (Fisher Chemical, Loughborough, UK) was optimal LC/MS grade. Deionized water from an Aqua Purificator G7749 (Miele, Gütersloh, Germany) was used for the preparation of all solutions. The working solutions were prepared fresh every morning. The model sample solutions contained 10^{-7} M or 5 × 10^{-9} M of the analytes dissolved in the leading electrolyte (LE). Drinking tap water (Brno city, Czech Republic) and river water (taken from Svratka and Svitava Rivers in Brno and from the Brno Reservoir) were used without any pretreatment.

2.2. Instrumentation

The experiments were performed using a 7100 CE system (Agilent Technologies, Waldbronn, Germany). A bare fused-silica capillary of 100 μm id and 100 cm length (Agilent Technologies) was installed in the cartridge and kept at constant temperature of 20 °C. The samples were injected by pressure (20 or 50 mbar). The capillary was flushed and filled with the terminating electrolyte (TE) and the LE was placed into the inlet vial. Due to application of voltage of opposite (positive at inlet) polarity, anionic electromigration of the analyte zones proceeded in the opposite direction, but both the high speed of the electroosmotic flow and the ESI suction ensured transport of the zones of interest towards the detector. Neither the velocity of the electroosmotic flow nor that of ESI suction were measured as it was not necessary to know their exact values. Their overall constant magnitude was monitored by watching the repeatability of detection times which was very good in all experiments (RSD values 1–3%). All separations were performed using a running voltage of 20 kV. No additional pressure was applied to the capillary.

The CE-MS coupling was done via ESI using a commercial CE-ESI-MS interface (Agilent Technologies, Waldbronn, Germany). A coaxial flow of sheath liquid, composed of 5 mM acetic acid and 3 mM base (ammonium hydroxide, TEA or ETA) in 50% (v/v) methanol, was provided during analyses. It was supplied via a splitter in a system based on a 1200 series isocratic pump (Agilent Technologies, Waldbronn, Germany) at a flow rate of 8 μL/min. The 6130 single quadrupole mass spectrometer (Agilent Technologies, Waldbronn, Germany) was operated in both scan and selected ion monitoring (SIM) modes, using the following operation parameters: capillary voltage 3500 V negative; nebulizer pressure 10 psi; drying gas flow 10 L/min; drying gas temperature 200° C. Negative molecular ions $[M-H]^-$ were monitored in two independent channels at a fragmentor voltage of 100 or 130 V. Nitrogen gas was supplied by an NM32LA generator (Peak Scientific, Frankfurt, Germany). The CE and MS instruments were controlled and data acquisition performed by ChemStation software (Agilent, Waldbronn, Germany).