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Can neutral analytes be concentrated by transient isotachophoresis in micellar electrokinetic chromatography and how much?

Magdalena Matczuk[a], Lidia S. Foteeva[b], Maciej Jarosz[a], Markus Galanski[c], Bernhard K. Keppler[c], Takeshi Hirokawa[d], Andrei R. Timerbaev[b,c,*]

[a] Chair of Analytical Chemistry, Faculty of Chemistry, Warsaw University of Technology, Warsaw, Poland
[b] Vernadsky Institute of Geochemistry and Analytical Chemistry, Russian Academy of Sciences, Moscow, Russian Federation
[c] Institute of Inorganic Chemistry, University of Vienna, Vienna, Austria
[d] Graduate School of Engineering, Hiroshima University, Higashi-hiroshima, Japan

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ABSTRACT

Transient isotachophoresis (tITP) is a versatile sample preconcentration technique that uses ITP to focus electrically charged analytes at the initial stage of CE analysis. However, according to the ruling principle of tITP, uncharged analytes are beyond its capacity while being separated and detected by micellar electrokinetic chromatography (MEKC). On the other hand, when these are charged micelles that undergo the tITP focusing, one can anticipate the concentration effect, resulting from the formation of transient micellar stack at moving sample/background electrolyte (BGE) boundary, which increasingly accumulates the analytes. This work expands the enrichment potential of tITP for MEKC by demonstrating the quantitative analysis of uncharged metal-based drugs from highly saline samples and introducing to the BGE solution anionic surfactants and buffer (terminating) co-ions of different mobility and concentration to optimize performance. Metallodrugs of assorted lipophilicity were chosen so as to explore whether their varying affinity toward micelles plays the role. In addition to altering the sample and BGE composition, optimization of the detection capability was achieved due to fine-tuning operational variables such as sample volume, separation voltage and pressure, etc. The results of optimization trials shed light on the mechanism of micellar tITP and render effective determination of selected drugs in human urine, with practical limits of detection using conventional UV detector.

1. Introduction

Over the three decades of continuous methodological developments, micellar electrokinetic chromatography (MEKC) has reached the status of a routine analytical tool for a great variety of substances [1–3]. This is due largely to systematically addressing the challenge of limited concentration sensitivity of MEKC by implementing an array of preconcentration techniques, operating in-line, i.e., within the same capillary where separation takes place [4,5]. Of these techniques, sweeping and its modifications (or combinations) offer perhaps the most remarkable improvements in detection sensitivity [6]. The analytical potential of analyte stacking by virtue of sweeping phenomenon is exceptionally high for samples with low-to-moderate conductivity, comparable to that of the background electrolyte (BGE) solution. However, such analytical conditions are not the occurrence for a variety of applications, in particular, routine clinical screening of biological samples, and contradictory results have been observed when sweeping was attempted under inhomogeneous electric field conditions, including a frequent case of high-salt sample matrices (see Ref. [7] and references therein). A theoretical analysis performed by Pyell and her coworkers [7] revealed the event of a strong deviation of the sweeping efficiency from the theoretically predicted value. Unexpectedly high enrichment factors were ascribed by the authors to a transient isotachophoresis (tITP) state induced by the presence in the sample of a salt whose co-ion (the ion with the same charge polarity as micelles) could act as a leading ion. (It goes without saying that to establish the tITP setting the electrophoretic mobility of micelles has to be bracketed by the mobility of a comparatively slow, preferably BGE co-ion.) Assuming that an isotachophoretically stacked zone of micelles migrates through the sample zone, it would concentrate the analytes, presumably to a greater extent

* Corresponding author at: Vernadsky Institute of Geochemistry and Analytical Chemistry, Kosygin St. 19, 119991 Moscow, Russian Federation.
Tel.: +7 495 9397035; fax: +7 495 9382054.
E-mail address: andrei.timerbaev@univie.ac.at (A.R. Timerbaev).

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than at ordinary sweeping conditions. Yet before this fundamental work, an apparently alike observation was made by Quirino who studied neutral analyte focusing by micelle collapse [8]. Later on, Foteeva et al. [9] simulated micelle stacking in MEKC and experimentally verified the possible contribution of tITP to analyte enrichment.

Despite the fact that the underlying principles of tITP applied to analyzing saline samples are well codified [10,11] and the method appears to be feasible to concentrating neutral analytes in MEKC, there is so far only a single report dealing with a biosample traced in the literature. Ren et al. [12] demonstrated that the tITP focusing could be the reason of extremely high peak efficiency for 3-nitrotyrosine introduced into a MEKC system in urinal matrix. This unwelcome situation is not only because micellar stacking is inherently a multistep focusing process [7,9,13,14], governed by a mixed mechanism that is intricate to comprehend. At the time of writing, there was no systematic study on the effects of BGE composition but on the concentration of sodium chloride in the sample solution tested though with a limited number of analytes [7,9]. In this regard, it should be mentioned that a high concentration of the leader, while a prerequisite for tITP, cannot be considered as a resourceful factor for enhancing the enrichment power, as destacking via electrodispersion inevitably aggravates the focusing of micelles.

Research described in the present paper was intended to fill the gap in experimental assessment of micellar tITP. To address this need, the nature and concentration of anionic surfactant, the mobility and concentration of the terminating co-ion, sample volume, among the other variables affecting the stacking efficiency, are examined for optimization of MEKC analysis of uncharged metallodrugs. Special focus is placed on verifying the occurrence of micelle stacking when a MEKC system is free of the leading or terminating anion, or the BGE contains the surfactant below its critical micelle concentration. To demonstrate the analytical relevance of the optimized tITP–MEKC system, two investigational anticancer metallodrugs were selected for quantification in urine. Such analyses present an important component of a successful lead-drug candidate selection program with respect to the determination of drug clearance via renal excretion.

2. Experimental

2.1. Instrumentation

All experiments were performed with a CAPEL 105M (Lumex Instruments, St. Petersburg, Russia) or an Agilent CE^{3D} system (Waldbronn, Germany). Polyimide-coated fused-silica capillaries of 75 μm I.D. (CM Scientific Ltd., Silsden, UK) with a length of 60 cm, 50 cm to the detector, were initially conditioned with 1 mol/L NaOH for 15 min, followed by water (10 min) and finally with the BGE for 15 min.

2.2. Chemicals

Cisplatin [*cis*-diamminedichloridoplatinum(II)] was purchased from Sigma. Oxaliplatin, *cis*-[(1R,2R)-cyclohexanediamine-*N*,*N*′] oxalato(2-)-*O*,*O*′]platinum(II), (OC-6-33)-dichlorido(ethane-1,2-diamine)bis{(4-propylamino)-4-oxobutanoato}platinum(IV) or $PtCl_2(eda)X_2$, (OC-6-33)-dichlorido(ethane-1,2-diamine)bis{(4-propyloxy)-4-oxobutanoato}-platinum(IV) ($PtCl_2(eda)Y_2$), and tris(8-quinolinolato)gallium(III) ($GaOx_3$) were synthesized at the University of Vienna. Formulas of the compounds are presented in the Supplementary material (Fig. S1; note the octanol–water partition coefficients (log *P*) given in parentheses).

Surfactants tested in this study were SDS, sodium cholate (both from Sigma-Aldrich) and sodium deoxycholate (Fluka). Sodium chloride, sodium tetraborate, sodium dihydrogen phosphate, disodium hydrogen phosphate, and acetone were from Sigma-Aldrich. Glycine, HEPES, and MES were purchased from Sigma, sodium hydroxide, hydrochloric acid, and Sudan III—from Fluka.

2.3. Solution and sample preparation

All solutions were prepared using 18 MΩ/cm water (Milli-Q Direct, Millipore, France) and filtered through 0.45-μm membrane filters (Millipore) before use. Stock solutions of metal complexes were prepared separately in 100 mmol/L NaCl at 0.3 or 0.1 mmol/L. The acidity of buffer solutions (100 mmol/L) was adjusted to a desired pH with diluted sodium hydroxide or hydrochloric acid (except for the phosphate buffer). The phosphate buffer was prepared by combining 20 mmol/L NaH_2PO_4 and 20 mmol/L Na_2HPO_4 to adjust the pH to 7.4. BGEs for MEKC were prepared by dissolving the weighted amount of a surfactant in an appropriate volume of the respective buffer solution and diluting the mixture with water to achieve final buffer and surfactant concentrations in the range from 10 to 50 mmol/L and from 5 to 100 mmol/L, respectively. In experiments on monitoring the micelle migration, the hydrophobic dye, Sudan III (0.25 mmol/L), dissolved in a mixture of acetone (30% v/v) and 100 mmol/L NaCl, was used as sample.

Samples of urine from a healthy volunteer with informed consent was obtained in polyethylene tubes and analyzed during the day of collection. Prior to analysis, drug-free samples were centrifuged for 5 min (7000 rpm) and the supernatant was spiked with the appropriate amount of drug solution, diluted 10 times, and filtered through a 0.45-μm membrane filter. Blank solution was prepared similarly using 100 mmol/L NaCl instead of the drug solution. No special procedure was adopted for disposal of the used samples.

2.4. Experimental procedure

The capillary was flushed daily in the order of 1 mol/L NaOH (15 min), water (10 min) and BGE (15 min) by pressure. Between runs, the capillary was rinsed using the same three-step washing sequence (for 5, 3, and 5 min, respectively). Samples were introduced into the capillary hydrodynamically, by applying a pressure of 20–50 mbar for a designated time. All separations were performed with the capillary thermostated at 25 °C, by applying the voltage (as a positive potential to the inlet vial) and simultaneously the pressure (typically 5 mbar). Since the anticipated tITP state was created by the addition of a suitable terminating ion to the micellar BGE while the sample contained the leading ion (Cl^-), no special amendments to standard MEKC procedure were required. Electropherograms were recorded at 200–220 nm. The main steps involved in tITP–MEKC are presented in Fig. 1 and commented below.

3. Results and discussion

3.1. Mechanism of micellar tITP

The mechanism of analyte stacking by tITP in MEKC is still a matter of debate, being likely an issue preventing its practical acceptance. Our view on how this could be done is illustrated in Fig. 1. It is important to note that from previous work [9], it is uncertain whether the analytes drive into the stacked zone of SDS micelles or the latter migrates through the sample zone to capture analytes. To avoid such an ambiguity, a pressure-assisted