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Comparison of two low flow interfaces for measurement of mobilities and stability constants by affinity capillary electrophoresis–mass spectrometry

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ABSTRACT

Affinity capillary electrophoresis (ACE) is typically used for the determination of stability constant, K_{st} , of weak to moderately strong complexes. Sensitive detection such as mass spectrometry (MS) is required for extension of ACE methodology for estimation of K_{st} of stronger complexes. Consequently, an efficient interface for hyphenation of CE with MS detection is necessary. For evaluation of interfaces for electrospray ionization mass spectrometric (ESI/MS) detection in ACE conditions, potassium-crown ether complexation was used as model system. The effective mobilities of the crown ether ligands and the K_{st} of their potassium complexes were measured/determined by ACE-ESI/MS using two lab-made interfaces: (i) a sheathless porous tip CE-ESI/MS interface and (ii) a nano-sheath liquid flow CE-ESI/MS interface, and, in turn, compared with those obtained by ACE with UV spectrophotometric detection. Apparent stability constant of potassium-crown ether complexes in 60/40 (v/v) methanol/water mixed solvent, pH* 5.5, was about 1300 L/mol for dibenzo-18-crown-6, 1600 L/mol for benzo-18-crown-6 and 5200 L/mol for 18-crown-6 ligands, respectively. It was observed that electrophoretic mobilities from CE-MS experiments differ from reference values determined by UV detection by ~7% depending on the CE-MS interface used. Good agreement of CE-MS and CE-UV data was achieved for nano-sheath liquid flow interface, in which the spray potential and the CE separation potential can be effectively decoupled. As for sheathless porous tip interface, a correction procedure involving a mobility marker has been proposed. It provides typically only ca. 1% difference of effective mobilities and K_{st} values obtained from CE-MS data as compared to those received by the reference ACE-UV method.

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1. Introduction

Capillary electrophoretic (CE) techniques are valuable tools for the physico-chemical characterization of (bio)molecules and their interactions with a variety of ligands [1,2]. The major benefit of CE techniques with respect to other methods applied for K_{st} determination, such as HPLC [3], spectrophotometry [4], and

electrochemistry [5], is very low sample consumption (nanoliter volumes of (sub)millimolar analyte solutions per one analysis).

So far, several CE modes have been applied for the study of non-covalent interactions of compounds [6–8]. For the determination of the stability constants, K_{st} , of kinetically labile complexes, the mobility shift affinity capillary electrophoresis (ACE) is the most frequently used method among them [1,6,9]. The K_{st} determination is based on the measurement of the dependence of the effective electrophoretic mobility of one interacting partner (ligand, L) on the concentration of the other interacting partner (central atom/ion, M) present in the background electrolyte (BGE). The method is suitable for the determination of the K_{st} of weak to moderately strong complexes with sufficiently good accuracy and precision. The accuracy of the K_{st} determination by ACE has been improved by the utilization of nonlinear regression analysis for the data evaluation [10,11] and corrections of the migration times of the analyte zones to the constant temperature, viscosity and ionic

Abbreviations: 18c6, 18-crown-6 ether; ACE, affinity capillary electrophoresis; BGE, background electrolyte; b18c6, benzo-18-crown-6 ether; db18c6, dibenzo-18-crown-6 ether; DMSO, dimethyl sulfoxide; EOF, electroosmotic flow; ESI, electrospray ionization; HV, high voltage; MeOH, methanol; MS, mass spectrometry; p-I-BP⁺, 1-(4-iodobenzyl)pyridinium ion; SL, sheath liquid.

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strength [12–14]. Moreover, the recently released program, CEval [15], developed in Dubsky's group allows for fitting the CE records by the Haarrhoff-Van der Linde (HVL) function [16] and for the accurate determination of migration times [17] in several seconds.

Despite the improvements mentioned above, the application of ACE in a common CE apparatus with a UV absorption detector is still limited to the determination of a K_{st} lower than ca 10,000, which can be reliably determined by this method. The limitation originates from the relatively low concentration sensitivity of the commonly used UV detectors. The concentration of the ligand (L) has to be at least two orders lower than the concentration of the central metal ion present in the BGE for the accurate determination of K_{st} by ACE. This requirement is fulfilled for moderately strong complexes ($\log K_{st} < 4$). The determination of the K_{st} of stronger interactions requires the use of a lower concentration of the central ion added to the BGE [18]. In order to achieve at least two-order differences between the central-ion and ligand concentrations, the concentration of the injected ligand has to be lower as well. Therefore, the applicability of mobility-shift ACE is limited by the sensitivity of the detection technique used.

Apart from fluorescence detectors [19], also mass spectrometers (MS) are highly sensitive detectors compatible with CE [20]. In the last twenty years, several approaches for the online hyphenation of the CE to MS have been explored [21–25]. The developed interfaces differ in sensitivity, ruggedness and applicability.

Nowadays, the online CE-ESI/MS is applied in metabolomics [26], proteomics [27], food [28] and drug analysis [29], forensic analysis [30], enantioseparations [31], etc. In contrast, only a few papers deal with the determination of stability constants by CE–MS. The most recently released paper from Chen's group deals with the determination of stability constants by frontal-analysis CE–MS [32]. For the mobility-shift ACE-MS, the inductively coupled plasma MS (ICP-MS) [33] or electrospray-ionization MS (ESI-MS) with a common coaxial sheath-liquid flow interface [34] is usually used.

Sensitivity improvement is the main reason for CE-ESI/MS interface development. The first commercially available CE-ESI/MS interface with coaxial sheath liquid flow had low sensitivity mainly due to the relatively high flow rate of the sheath liquid required for stable electrospray. Later developed interfaces with modified designs require much lower (e.g. nano-sheath liquid flow interface [35] or micro flow-through vial [36]) or no (e.g. sheathless porous tip interface [37]) flow rates of the sheath liquid, which results in improved sensitivity.

The ability of the interface to control spray potential, E_{spray} , under changing separation conditions, e.g. varied separation voltage or BGE conductivity, was partially out of interest of the interface investigators. This problem seems to have been solved in interfaces utilizing a metallic ESI-emitter, such as micro flow-through vial [38] and coaxial sheath liquid flow CE-ESI/MS interfaces. In these cases, the E_{spray} is applied directly to the ESI emitter, which also serves as an electrode creating separation circuits. The actual value of E_{spray} is the value applied by high-voltage (HV) power supply. Nevertheless, in the cases of interfaces developed in Dovichi's and Moini's groups, the spray potential is applied indirectly through the conductive environment to the ESI emitter tip. Such an indirect connection may cause a potential drop between the potential applied by the HV power supply driving ESI (HV2) and the actual spray potential.

Flaherty et al. [39] have observed an increase of voltage in the sheath liquid reservoir (where the HV2 electrode is placed) under changing separation conditions for a nano-sheath liquid flow CE-ESI/MS interface. This increase enhanced the potential at the ESI-emitter tip, which decreased the detection sensitivity. The voltage in the sheath liquid reservoir was affected by the separation voltage in the case of highly conductive environment (highly conductive

BGE and/or wide capillary I.D.). This phenomenon was overcome by the modification of HV2 power supply.

The knowledge of the actual E_{spray} is essential for the calculation of the separation voltage, U_{sep} , which is an important parameter for the recalculation of time-dependent electropherograms to (more robust) mobility-dependent electropherograms [40], or for the determination of the physico-chemical characteristics of compounds (acid dissociation constants of weak electrolytes [41] or stability constants of complexes [42]).

The aim of this work was to compare the suitability of two highly sensitive CE-ESI/MS interfaces, namely slightly modified Dovichi's interface [43] and Moini's sheathless porous tip interface [37], for the determination of the stability constants of complexes by ACE with MS detection. On the other hand, frequently used sheath liquid flow interface like Agilent triple tube was not included in this study mainly due to its low compatibility with Thermo mass spectrometers. Instead, we studied the ability of the interfaces to keep spray potential under control with varied BGE conductivity. Moreover, the accuracy and precision of the stability constants determined using the interfaces were evaluated with respect to broader applicability. Stability constant of the model potassium ion complexes of UV-absorbing ligands dibenzo-18-crown-6 and benzo-18-crown-6 ether were determined by ACE-ESI/MS and the obtained data were compared to those obtained by ACE-UV. The determination of stability constant of non-UV absorbing potassium complex with 18-crown-6 demonstrates importance of MS detection in ACE studies of molecular interactions of UV-transparent species.

2. Materials and methods

2.1. Chemicals

Dibenzo-18-crown-6 ether (db18c6), 98%, benzo-18-crown-6 ether (b18c6), 98%, 18-crown-6 ether (18c6), 99%, methanol (MeOH), HPLC grade, dimethylsulfoxide (DMSO), $\geq 99.5\%$, potassium chloride, analytical grade, propiconazole, Pestanal ©, and acetic acid, $\geq 99.8\%$, were purchased from Sigma-Aldrich Ltd. (Prague, Czech Republic). Lithium hydroxide (analytical grade) was from Lachema (Brno, Czech Republic), sodium hydroxide and ammonia solution (both analytical grade) were from Penta (Prague, Czech Republic). The 1-(4-iodobenzyl)pyridinium chloride was synthesized according to the procedure described elsewhere [44]. Standard deionized water of 18 MOhm.cm was used.

2.2. ACE-UV measurements

ACE measurements with UV detection were performed on the Agilent CE 7100 (Agilent, Waldbronn, Germany) apparatus equipped with a diode array UV-VIS spectrophotometric detector and an active temperature-control system. Bare fused-silica capillaries of 50/375 μm I.D./O.D., 34.5/26 cm total/effective length, from Polymicro Technologies (Phoenix, AZ, USA) were used for the measurements.

A BGE was a 10 mM lithium acetate in the 60/40 (v/v) methanol/water mixture. Theoretical $\text{pH}^* = 5.5$ was calculated from $\text{p}K_a$ in respective solvent mixture. Same value of apparent pH was obtained using ordinary pH-meter intended for aqueous media. The concentration of potassium chloride in the BGE ranged from 0 to 8 mM. The measurements were performed at 20 °C. The sample mixture containing 2×10^{-5} M crown ethers, 1×10^{-4} M 1-(4-iodobenzyl)pyridinium chloride and 1×10^{-4} M propiconazole in BGE was introduced into the capillary hydrodynamically at 2 kPa for 10 s. Separation voltage was adjusted for each potassium chloride concentration in order to maintain the constant input power of 0.3 W. All measurements were performed in triplicates.

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