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Addition of reagents to the sheath liquid: A novel concept in capillary electrophoresis-mass spectrometry☆

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ABSTRACT

Conventional coupling of capillary electrophoresis with electrospray ionisation mass spectrometry typically relies on the use of a triaxial sheath-flow liquid interface to facilitate electrical contact and provide a stable electrospray. In this type of analysis, the use of additives in the sheath liquid itself can also be used to improve ionisation of analytes and even facilitate reactions between separation and detection steps (which we broadly term "sheath-flow chemistry"). In the present work, this concept is demonstrated using two types of sheath-flow reactions for CE coupled with quadrupole time-of-flight (Q-TOF) MS detection. Sheath liquid compositions containing deuterated solvents or DPPH• (2,2-diphenyl-1-dipicrylhydrazyl) stable free-radicals yield useful additional structural information for separated analytes. Investigations of fundamental physical and chemical characteristics of the sheath liquid coupling show their direct influence on the efficiency and some of the products of the respective reactions. For example, reducing the capillary internal diameter from 75 to 25 μm increased the relative abundance of fully deuterated ions detected by 63–65% (5 exchangeable hydrogens) using constant sheath-flow conditions. Addition of 0.05–0.2 mM DPPH• to the sheath liquid reduced the peak total ion count obtained for typical antioxidant species by 20 to >95% allowing strongly antioxidant species from mixtures to be readily identified and further studied. The presented approach allows a rapid and information-rich analysis to be performed with minimal reagent and sample consumption.

1. Introduction

The use of mass spectrometry (MS) as a detector for capillary electroseparation techniques has evolved from a rather exotic combination into a widely used hyphenated technique over the last 25 years [1]. Thereby, the crucial component for the realisation of such a combination is still the choice of the most appropriate interface for coupling the capillary electrophoresis (CE) instrument with the MS [2–4]. A wide range of interface types have been presented in the literature, but up to now (except for the porous tip interface developed by Moini [5] that might be commercialised in the near future) only the tri-axial sheath flow interface can be regarded as being widely used. Commonly listed advantages of this interface are its ease of use, robustness and the compatibility with commercially available electrospray (ESI) sources [1]. Despite the inherent ability to improve ionisation conditions by optimising the sheath liquid composition [6,7], the fact that the effluent from the separation capillary is substantially diluted by a sheath liquid is often seen as a drawback of this interface design [8]. On the other hand, one principal advantage of adding a sheath liquid that is already exploited routinely is the possibility of decoupling conditions for separation- from those for the ionisation process in the ESI source. So in both cases the optimum conditions (e.g. anionic for separation and cationic for detection) can be employed.

In this work we present a concept adding further functionality to the sheath liquid by performing so-called "sheath flow chemistry". This entails addition of one or more reagents to the sheath liquid leading to a chemical reaction upon mixing of the capillary effluent and the sheath liquid within the CE-MS interface. Up to now, such reactions have been employed in CE-MS only in very rare cases (e.g. the use of cationic ion-pair reagents was shown to be possible by Lin et al. [9] using a "post-reaction" strategy instead of the more favourable "pre-" and "on-column" approaches for the CE-MS determination of small anions). A more established example is hydrogen/deuterium (H/D) exchange employing

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deuterated solvents in the sheath liquid thereby allowing determination of the number of exchangeable protons within separated analyte molecules [10,11]. Online H/D exchange is principally useful for assaying molecules with low (<10) numbers of exchangeable hydrogens when utilised with CE-MS or HPLC-MS as the kinetics of H/D exchange are relatively fast. It is important for this type of analysis that the fully exchanged ion is observed in the peak mass spectrum as this is critical for simple structural confirmation. A further option for the use of "sheath flow chemistry" would be reactions for characterising the antioxidant activity of analytes where the ability of potential antioxidants (analytes) to scavenge free radicals is investigated.

Typically, antioxidant properties of individual compounds or mixtures (extracts) are tested using simple colorimetric assays which measure antioxidant scavenging ability towards a stable radical species [12]. Despite their popularity, the simplicity of such free radical assays is often overstated as reactions with phenolic compounds can proceed via hydrogen abstraction or electron transfer mechanisms [13] potentially yielding numerous reaction products. Therefore, quantitative colorimetric determination of the antioxidant activity might not be entirely robust as shown by several studies [14–16]. Even if these issues do not cause a serious error for these determinations and subsequent elucidation of structure–activity relationships, colorimetric assays alone cannot discriminate which particular compounds within an extract containing potentially numerous antioxidants exhibit the highest activity. For these reasons, the coupling of separation techniques with antioxidant assays is one option for in-depth investigation of antioxidant activity [17]. Focusing on HPLC separations, such studies entail use of a second pump for post column addition of a solution containing a stable free radical that is mixed in a reaction coil with the column effluent immediately prior to detection [18,19]. The information obtained from these assays can be further increased when, instead of colorimetric detection, MS detection is employed thereby detecting products formed in the reaction of "free radical reagent" with the analytes. In the case of CE-MS, a similar approach can be followed by adding appropriate concentrations of the free radical reagent to the sheath liquid.

In the present work we investigate the potential of "sheath flow chemistry" in CE-MS using two selected example reactions namely H/D exchange and reaction with stable free radicals as commonly employed in antioxidant assays of phenolic compounds. The straightforward H/D exchange reaction is studied in some detail to investigate fundamental parameters of sheath-flow reactions using CE-MS, while the novel application of an online antioxidant assay demonstrates how this approach can be used for other practical applications. In both cases, chemical reactions are facilitated upon mixing of a sheath liquid containing the appropriate reagent with the analytes present in the effluent from the CE capillary. To ensure greater confidence in identifying reaction products and isotopic abundance accuracy, high resolution Q-TOF MS detection is employed.

2. Materials and methods

2.1. Materials

Ammonium hydroxide and the HPLC grade solvents (methanol and 2-propanol) were obtained from VWR (Vienna, Austria). Ammonium acetate, sodium phosphate and sodium dihydrogen phosphate were obtained from Merck (Darmstadt, Germany). 2,2-diphenyl-1-dipicrylhydrazyl (DPPH•), caffeic acid, (+)-catechin hydrate, gallic acid, gentisic acid, morin hydrate, protocatechuic acid, 2-pyrocatechuic acid, quercetin, β-resorcylic acid and γ-resorcylic acid were obtained from Sigma-Aldrich (Vienna, Austria). Deuterium oxide was obtained from C.E. Saclay (Gif sur Yvette, France) and methan(ol-d) from Witega (Berlin, Germany). All chemicals used were analytical grade unless otherwise specified. 18 MΩ cm purified water was from a Milli-Q Reference A+ purification system from Merck Millipore (Vienna, Austria).

2.2. Preparation of sheath liquids

Deuterated sheath liquids were prepared using D_2O and methan(ol-d). Low concentrations (<1%, v/v) of concentrated ammonium hydroxide (non-deuterated) were added to improve ionisation in the MS. Sheath liquid was prepared fresh daily.

Stock solutions of DPPH• were prepared fresh daily at a concentration of 1 mM in methanol or 2-propanol. The sheath liquid composition for the DPPH• assay contained 80% (v/v) of the organic/DPPH• phase with 20% (v/v) of aqueous ammonium hydroxide solution. The final concentration of DPPH• was between 0.05 and 0.2 mM and the final concentration of ammonium hydroxide was 0.01% (v/v). It should be noted that the concentration of ammonium hydroxide in the final sheath liquid composition was required to be minimal to avoid decomposition of the DPPH•. Typically, the visual appearance (colour and transparency) of the sheath liquid was found to be consistent for more than 24 h in the presence of 0.01% ammonium hydroxide, but sheath liquid compositions were diluted daily to ensure a consistent concentration of the free radical scavenger.

2.3. CE instrumentation

CE experiments were carried out using an Agilent ^{3D}CE capillary electrophoresis instrument (Waldbronn, Germany), equipped with a deuterium UV lamp and diode array detector (190–600 nm). Agilent Chemstation system software was used for data acquisition and instrument control. Bare fused silica capillaries were purchased from Polymicro Technologies (Phoenix, AZ, USA). Capillaries were conditioned by flushing with 0.1 M sodium hydroxide and then water for 10 min each prior to measurements. Capillaries were flushed for 5 min with BGE between runs. This pre-conditioning protocol was applied for all CE and CE-MS measurements.

2.4. EOF measurements

Electroendoosmotic flow (EOF) values for fused silica capillaries were measured following the method of Williams and Vigh [20]. Briefly, measurements were made using background electrolytes consisting of 10 mM aqueous sodium phosphate adjusted to the relevant pH values (8.52, 5.45, and 2.91). Caffeine dissolved in high purity water was used as the EOF marker. Capillaries used for EOF measurements were all of 36.6 cm total length (28.1 cm effective length) and UV detection carried out at 210 nm.

2.5. CE-MS analysis

The CE instrument was coupled to an Agilent 6510 Q-TOF mass spectrometer equipped with an electrospray ionisation (ESI) source and an Agilent G1607A coaxial sprayer (all from Agilent). Analyses were performed in the negative ionisation mode. Nitrogen was used as drying gas at a temperature of 250 °C and a flow rate of 4 L/min. The nebuliser pressure was 8 psi. The sheath liquid was delivered via a syringe pump (KD Scientific, MA, USA) using a 500 μL Hamilton Gastight syringe (Unilab Technologies GmbH, Innsbruck, Austria) at 4 μL/min. The MS capillary voltage was 3750 V and the fragmentor was set to 140 V. The scanning mass range was from m/z 70 to 800 with an acquisition rate of 3 spectra/s. For extraction of ion electropherograms, a tolerance of ±200 ppm was used. It