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Performance of an electrospray-interfaced thermospray ion source in hyphenated techniques

R.A.M. van der Hoeven, B.A.P. Buscher, U.R. Tjaden*, J. van der Greef

Division of Analytical Chemistry, Leiden/Amsterdam Center for Drug Research, Leiden University, P.O. Box 9502, 2300 RA Leiden, Netherlands

Abstract

The performance of a custom-made electrospray interface coupled with a thermospray ion source is described, using tetraalkylammonium salts and lysozyme as test compounds. Optimal conditions are created by using a heated sampling capillary, increasing the pumping capacity in the ion source and by changing the shape of the repeller to conical. Under these conditions the performance of the interface, with respect to sensitivity, is comparable with previously described systems using low flow-rates. However, due to the use of a heated sampling capillary flow-rates up to 100 μl/min can be used. Examples of coupling electrospray with capillary electrophoresis and micro-LC in negative ionization mode are given. As test compounds inositol phosphates and acyl-coenzyme A compounds are used.

1. Introduction

Since 1984, when electrospray ionization (ESI) in combination with mass spectrometry was first reported by Yamashita and Fenn [1] and Aleksandrov et al. [2] simultaneously, ESI has become a powerful tool in detecting intact multiple protonated molecules up to M_r 60 000 by the use of constant infusion of protein containing solutions. Using low flow-rates makes it interesting to couple ESI with capillary electrophoresis (CE) [3] and micro-LC [4]. The compounds of interest vary from small ionic species, like human growth hormones, to polypeptides and proteins. Interfacing in hyphenated techniques has resulted in the development of various types of ESI interfaces. The ideal system should cover the sensitivity, efficiency, stability and applicability of all different systems. Unfortunately, such a device does not exist. In the first generation ESI interfaces, like the Whitehouse–Fenn type [5], the flow-rate is limited to a few microlitres per minute with a rapid decrease in signal when increasing the flow-rate. Working in negative ionization mode is difficult, due to corona discharge. The use of chlorinated solvent [6] or oxygen as a bath gas [7] can partly overcome this problem, giving a more stable signal. Working at higher flow-rates, especially using aqueous solvents, limits the use of both microbore and conventional LC coupled with ESI. Part of this problem can be solved by working with a nebulizing gas, forming a so-

* Corresponding author.

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called ionspray [8], or when using aqueous solvents to suppress the corona discharge with sulphur hexafluoride [9]. A bad response at higher flow-rates can be attributed to insufficient drying of the formed electrospray, due to the increasing droplet size, as described by Vestal [10]. Actually, there are two ways to solve this problem: (1) increasing the drying capacity by heating the spray with a heated sheath gas [11] and (2) using a heated sampling capillary, as shown by Chowdhurry et al. [12]. Nowadays, ESI interfaces are commercially available which more or less overcome the flow-rate problem.

As the ESI source may be a simple metal capillary at elevated voltage relative to a counter electrode, we investigated the possibilities of coupling such a device to a TSP ion source which acts as an intermediate pressure region. A closely related approach was recently described by Jackett and Moini [13], using a flanged ESI adapter mounted to the TSP ion source, in-line with the quadrupole axis.

We describe the use of the original configuration of the TSP ion source, replacing the TSP vaporizer probe by a flanged heated stainless-steel capillary, which fits in the ion source, implying that the heated capillary is mounted at a 90° angle with respect to the quadrupole axis. A low positive or negative voltage at the repeller, depending on the ionization mode, pushes ions into the mass spectrometer. In the configuration tested, the shape of the repeller has been changed to conical, to obtain better sensitivity in the higher mass range. The use of a heated capillary makes it possible to achieve a good performance in positive as well as in negative ionization mode, even at flow-rates of 100 μl/min. Although this is a simple and inexpensive ESI interface, sensitivities are comparable to other ESI interfaces.

Examples of both high- and low-molecular-mass compounds at high and low flow-rates are shown. The coupling is illustrated with both CE and micro-LC. For this purpose, examples are chosen which include chemically and thermally unstable compounds, such as inositol phosphates in CE–MS mode and some acyl-coenzyme A compounds using micro-LC, both examples in negative ionization mode.

2. Experimental

2.1. Interface

All experiments were performed on a Finnigan MAT (San Jose, CA, USA) TSQ-70. The custom-made electrospray interface fits in the thermospray ion source as is shown in Fig. 1.

Depending on the ionization mode, the ESI needle assembly is kept at either −3 or +3 kV towards the heated sampling capillary, which is kept at earth potential. The TSP ion source is also kept at earth potential. The heated capillary consists of a 1/16 in. O.D., 0.5 mm I.D. stainless-steel capillary with a length of 0.09 m, which fits in the ion source by means of a Vespel connector. The sampling capillary together with the heater unit is connected with a nut to a flange and is made as a removable probe. The capillary can be heated up to 300°C. The working temperatures of the heated capillary and the ion source during the experiments were 175 and 150°C, respectively. The best operation was performed by using a conical-shaped repeller and by replacing the original rotary pump (UNO 16, 16 m^3/h, Balzers, Asslar, Germany) by a pump

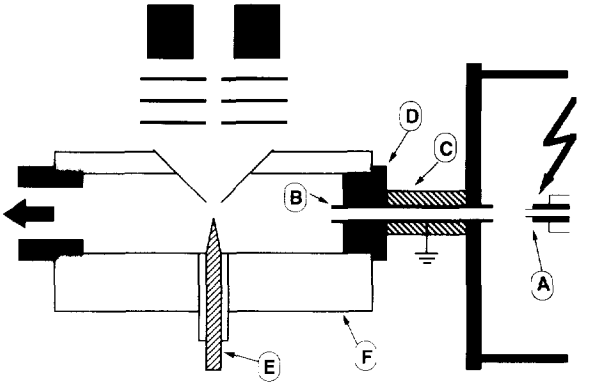

Fig. 1. Schematic diagram of the custom-made electrospray interface: (A) electrospray needle assembly, (B) sampling capillary, (C) heater assembly, (D) vespel connector, (E) conical-shaped repeller, (F) thermospray ion source block.

with higher capacity (E2M28, 28 m^3/h, Edwards, UK).

2.2. Characterization

For the characterization of the custom-made ESI interface in the low mass range a mixture of tetraalkylammonium salts was used, while in the high mass range lysozyme was applied. All chemicals were dissolved in a mixture of methanol and water containing 1% acetic acid (80:20, v/v).

To characterize the use of higher flow-rates, tetraalkylammonium salts were used, with and without the use of nitrogen as a nebulizing gas.

In the constant-infusion mode experiments were performed with a syringe pump (Harvard Apparatus, South Natick, MA, USA). Flow injection experiments (FIA), using tetraoctylammonium bromide (TOA) (M_r 466.4) as test compound, were done at a flow-rate of 1 μl/min using a Phoenix 20 CU syringe pump (Carlo Erba, Rodano, Italy) in combination with a Valco CI4W micro-injector with an injection volume of 60 nl (Valco Instruments, Houston, TX, USA). Methanol–water (80:20, v/v) containing 1% acetic acid was used as a liquid sheath flow during all experiments to serve as an electrical contact.

2.3. Micro-LC–MS

For the coupling of micro-LC with MS (for the determination of acyl-coenzyme A compounds), a Phoenix 20 CU syringe pump was used to deliver the solvent. A C_8 slurry-packed fused-silica column, 100 mm × 220 μm I.D., was directly connected to a Valco CI4W micro-injector with an injection volume of 60 nl. To eliminate dead volumes, a 170 μm O.D., 75 μm I.D. fused-silica capillary was inserted in the column towards the glass-wool frit. The 170 μm O.D. fused-silica fits in the needle assembly. The mobile phase consisted of a mixture of acetonitrile–0.05 *M* ammonium/water (10:90, v/v), at a flow-rate of 2 μl/min in combination with a sheath flow, delivered by a syringe pump, of 2 μl/min of acetonitrile–0.05 *M* ammonium acetate/water (90:10, v/v).

2.4. CE–MS

For the coupling of CE to MS, the fused-silica capillary, O.D. 170 μm, I.D. 100 μm and l = 0.85 m, was inserted into the stainless-steel needle assembly in such a way that the tip of the fused-silica slightly sticks out of the stainless-steel needle. For the best performance the polyimide coating has been removed from the tip of the fused-silica protruding from the needle assembly.

A programmable injector (Prince, Lauerlabs, Emmen, Netherlands) was used to control the injection and the high voltage. The working voltage was −28 kV towards the needle assembly at −3 kV. To eliminate the electroosmotic flow (EOF), the fused-silica was coated with polyacrylamide [10], and also pressure of 10 mbar was applied. As CE buffer a mixture of methanol and ammonium acetate in water (10 m*M*, pH 5.0) (10:90, v/v) was used. The sheath flow had the same ionic strength and was composed of methanol and ammonium acetate in water (100 m*M*, pH 5.0) (90:10, v/v) delivered by a syringe pump with a flow-rate of 2 μl/min.

2.5. Chemicals

Methanol, acetonitrile and acetic acid (Baker, Deventer, Netherlands) were of HPLC grade. Water was purified with a Milli-Q apparatus (Millipore, Bedford, MA, USA). Ammonium acetate was purchased from Merck (Darmstadt, Germany). Tetraalkylammonium (TAA) salts were obtained from Aldrich (Steinheim, Germany). Coenzyme A derivatives and lysozyme came from Sigma (St. Louis, USA) and inositol phosphates from Perstorp (Perstorp, Sweden).

3. Results and discussion

The aim of this study was to develop an ESI interface that could compete with commercially available ESI interfaces regarding sensitivity and

costs, and that should cover a wide range of applicable flow-rates. The basis of our design was a thermospray (TSP) ion source. In fact, the original TSP probe has been replaced by a flange with a sampling capillary with 500 μm I.D., separating the ambient-pressure region from the low-pressure region. The ions are extracted out of the ion source at a 90° angle with respect to the quadrupole axis. Changing from TSP to ESI takes less than one hour.

As a starting point an unchanged TSP ion source, in combination with the original 16 m^3/h rotary pump, was chosen. Replacement with a rotary pump with higher capacity (28 m^3/h) resulted in an improved performance, which was tested with TAA salts. The increased pumping speed had little influence on the optimum repeller voltage, but ion intensity increased due to reduced ion-source pressure, and consequently resulted in a reduced energy distribution of the expanded ions out of the sampling capillary. The shape of the repeller did not affect sensitivity for TAA ions. However, in the high mass range using lysozyme as a test compound, far better results could be achieved with a conical-shaped repeller instead of a flat one. A five-fold increase in sensitivity was reached. The conical shape results in a totally different field in the expanded jet, capable of extracting higher-mass molecules more efficiently. In Fig. 2 the mass spectrum of 1 pmol lysozyme consumed, infused at 1 μl/min, is shown. The molecular mass determined after deconvolution was 14 303 ± 3, which is in close agreement with the theoretical value.

The satellite peaks can be identified as phosphate or sulfate clusters, which has been described earlier by Chowdhury et al. [15]. With this setup, the limit of detection for lysozyme could be estimated as 100 fmol consumed. This is in agreement with the sensitivity obtained with the Whitehouse–Fenn interface [5], which has been in use for a few years in our laboratory.

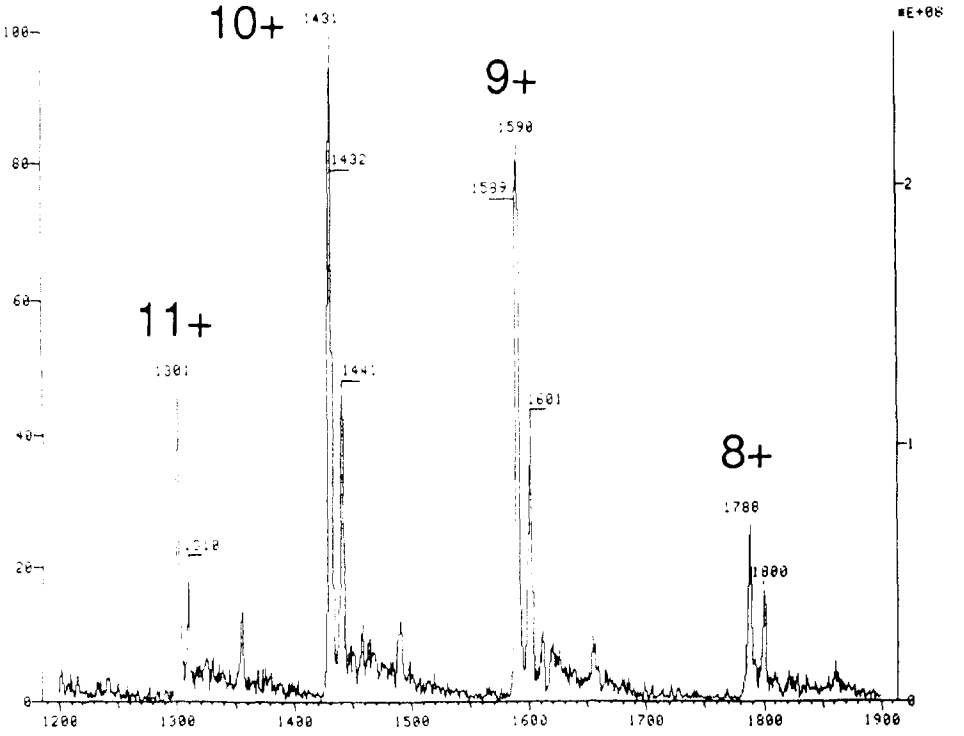

Fig. 2. Electrospray mass spectrum of lysozyme obtained with constant infusion of 1 pmol/μl in methanol–water (80:20, v/v) +1% acetic acid.

Thermal-induced dissociation of multiply protonated peptides, as shown by Rockwood et al. [16], has not yet been observed. This is probably due to the limited heat capacity of the used sampling capillary. Another way of inducing fragmentation in ESI is known as nozzle-skimmer-induced fragmentation [17]. Using the same ion-source configuration, only in TSP mode, increasing the repeller voltage is used to get structural information. Until now it was not possible to observe the same phenomenon in the ESI mode. This subject is still under investigation at the moment.

At higher flow-rates the cone form at the tip of the needle assembly will be changed drastically, but due to Rayleigh instabilities, small droplets will be formed. Vestal [10] made a prediction of the droplet diameter to be proportional to the 2/3 power of the flow-rate, with diameters on the order of 1–2 μm at 1 μl/min and about 100–200 μm at 1 ml/min. The drying capacity of the surrounding air is insufficient for complete dissolvation of the bigger droplets, which consequently results in a decrease of the signal. Methods to overcome this problem are mentioned in the literature [11,12].

By means of a heated capillary, the useful flow-rate can already be extended to 100 μl/min. As a consequence, the signal will be reduced by a factor of four. This was tested with tetraoctylammonium bromide (TOA) as a test compound in constant-infusion mode, as well as in FIA experiments.

Using nitrogen as nebulizing gas, the signal is more stable using flow-rates of 25 μl/min and higher; also the signal even increases in comparison with no nebulizing gas. With a liquid flow-rate of 25 μl/min, using nebulizing gas, subsequent injections of 215 fmol TOA, scanning from M_r 100 to 500, gave a standard deviation of 5%, at a signal-to-noise ratio of 10.

3.1. Application to micro-LC–MS

Acyl-coenzyme A compounds are highly polar, and chemically and thermally unstable, so soft-ionization techniques are required for identification. Recently, Norwood et al. [18] and Millington et al. [19] described a method for determination of several acyl-coenzyme A compounds using continuous-flow (CF) fast atom bombardment (FAB)-MS in the positive ionization mode. The mass spectra show, besides the protonated molecule, a lot of fragmentation. The reported detection limits are in the range of 50–100 pmol. We investigated the possibilities of using the described ESI in combination with micro-LC for the determination of some acyl-coenzyme A compounds in negative ionization mode. No attempts have been made to optimize the system with respect to eluent composition for separation and mass spectrometric sensitivity. Acetyl-coenzyme A (A-CoA) and 3-hydroxy-3-methylglutaryl-coenzyme A (MG-CoA) have been used as test compounds. These compounds can only be separated using low percentages of organic solvent, which are in fact less favourable conditions for ESI. Application of a sheath flow with a high percentage of organic solvent enables the detection of A-CoA and MG-CoA at low picomolar levels, as is illustrated in Fig. 3.

Detection is based on deprotonated and doubly deprotonated molecules. Under the above-mentioned conditions, doubly deprotonated molecules are favoured. The optimal repeller voltage is the same for both species. No thermal fragmentation has been observed under these conditions, even not while heating the sampling capillary up to 250°C. MS–MS measurements should be used to obtain structural information.

Optimization of separation and mass spectrometric sensitivity of the acyl-coenzyme compounds is still under investigation, using micro-LC and pseudo-electrochromatography (PEC) [20]. The separation efficiency in micro-LC can be improved by changing the pH in combination with the application of gradient elution, as already described by Norwood et al. [18].

3.2. Capillary electrophoresis–mass spectrometry

The described interface allows the combination with extremely low flow-rate separation techniques as is demonstrated in the following example.

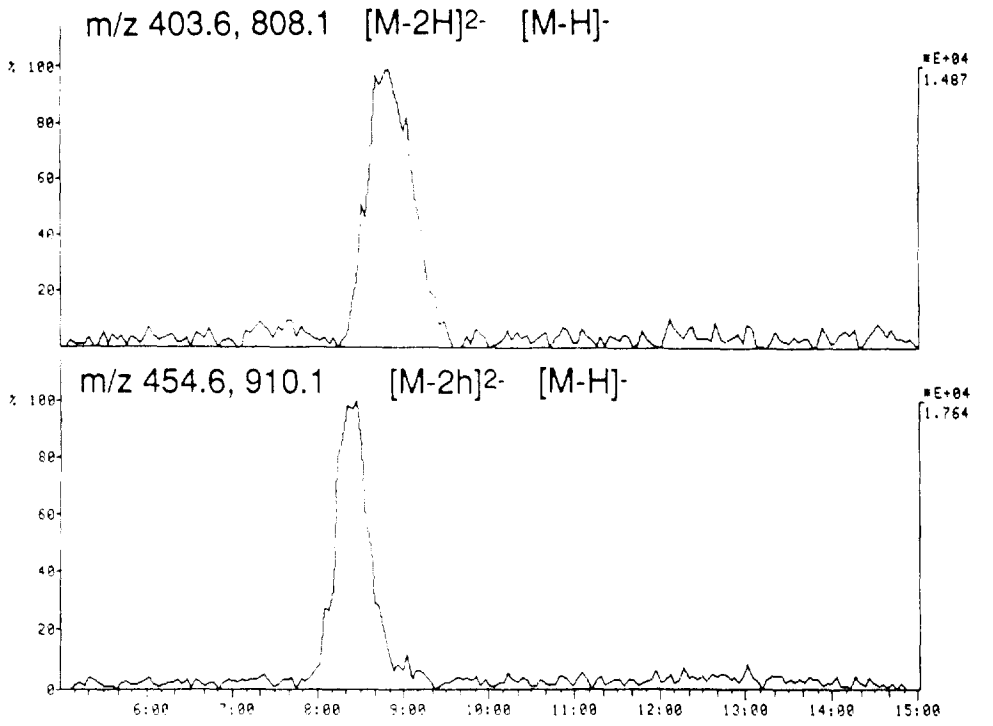

Fig. 3. Micro-LC–MS of 5 pmol injected each of A-CoA and MG-CoA separated on a C_8 slurry-packed column, using acetonitrile–0.05 *M* ammonium acetate in water (10:90, v/v) as eluent.

Capillary electrophoresis is a suitable technique for the separation of underivatized inositol phosphates at low pH, due to their strong ionic character. Mass spectrometric detection of inositol phosphates is described by Sherman et al. [21] and Walton and Hughes [22], using static FAB-MS, with detection limits in the nanomolar range. It has been demonstrated that mass spectrometry is a reliable and sensitive method of detecting inositol phosphates in the negative ionization mode using capillary electrophoresis [23]. In the configuration with the ESI needle assembly acting as cathode at −3 kV towards the anode at −28 kV, electromigration and the electroosmotic flow (EOF) are in opposite directions. Suppression of the EOF was achieved by coating the fused-silica capillary with polyacrylamide [14].

Preferably, the CE buffer and the sheath liquid should have the same composition. Nevertheless, hardly any signal could be detected in the negative ionization mode using a buffer composition of methanol–ammonium acetate in water (10 m*M*, pH 5.0) (30:70, v/v). Constant-infusion experiments showed that best sensitivity for inositol phosphates could be obtained with percentages of organic solvents of 80–100%, observing deprotonated molecules. Therefore, it was decided to keep the percentage organic solvent in the CE buffer as low as possible and in the sheath liquid as high as possible, while maintaining the ionic strength the same in both cases. Without any problem concerning stability and sensitivity, buffer compositions as mentioned in this study could be used. In Fig. 4, the electropherogram of a mixture of inositol phosphates is given. In this case 9 pmol of each component is injected. The differences in response factors of the inositol phosphates can be explained by the fact that mass spectrometric

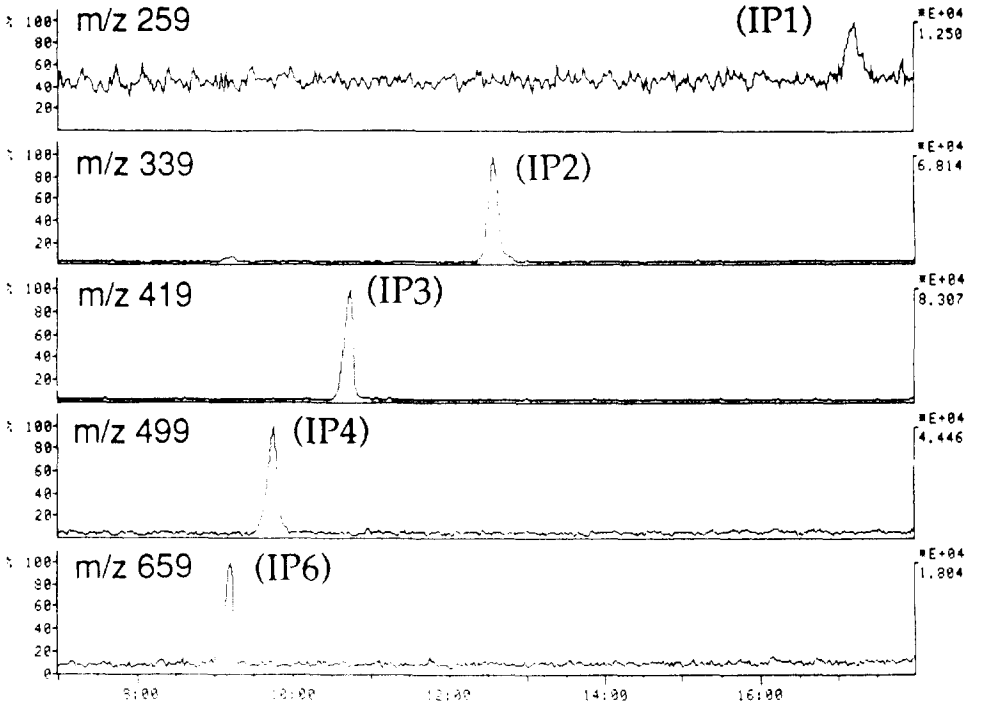

Fig. 4. Electropherogram of a test mixture of inositol phosphates. Amount injected: 9 pmol of each compound.

detection occurred in the MID mode, in which procedure only the singly charged ions were involved.

4. Conclusions

The custom-made electrospray interface in combination with a thermospray ion source offers sensitivities comparable with the Whitehouse–Fenn ESI interface, used in our laboratory, with respect to low flow-rates for low, as well as, high molecular masses. Extraction of ions out of the ion source, by means of a conical-shaped repeller, at a 90° angle with respect to the quadrupole axis, is quite effective. Until now no thermal-induced or repeller-induced fragmentation is observed.

Additionally, flow-rates up to 100 μl/min can be used without severe loss of signal, due to the heated sampling capillary.

Working in negative ionization mode is not affected by corona discharge. Therefore, the interface is flexible for coupling CE, micro-LC and LC with split. This is illustrated by the coupling of CE–MS for detection of inositol phosphates, obtaining low picomolar detection limits, and micro-LC–MS for determination of intact acyl-coenzyme A compounds.

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