

Easy Ambient Sonic-Spray Ionization-Membrane Interface Mass Spectrometry for Direct Analysis of Solution Constituents

Renato Haddad,[†] Regina Sparrapan,[†] Tapio Kotiaho,^{*,‡} and Marcos N. Eberlin^{*,†}

ThoMSon Mass Spectrometry Laboratory, Institute of Chemistry, State University of Campinas, 13084-971, Campinas SP, Brazil, and Faculty of Pharmacy, Division of Pharmaceutical Chemistry, and Laboratory of Analytical Chemistry, Department of Chemistry, University of Helsinki, Helsinki, Finland

Using a cellulose dialysis membrane and aqueous solutions of common drugs as a proof-of-principle example, we demonstrate that solid but permeable and flexible membranes can be used as interfaces for the direct analysis of solution constituents via easy ambient sonic-spray ionization mass spectrometry. This new combination of MS techniques, herein termed EASI-MIMS, promotes droplet pick up of the analyte from the external surface of the membrane from where the analyte has selectively permeated for proper mass spectrometry characterization and quantitation. Possible application of EASI-MIMS such as the environmental analyses of effluents, on-line monitoring of fermentation and biotransformations and on-line pharmacokinetic blood analysis are discussed.

The efficient desorption, ionization, and further characterization via mass spectrometry of analytes performed directly from their natural matrixes via a fully “nonsample prep” procedure, under atmospheric pressure and at room temperature, is certainly one of the most-welcomed advances of modern mass spectrometry. This previously unimaginable feature of MS has been made possible recently with the introduction of a variety of new techniques such as desorption electrospray ionization,¹ direct analysis in real time,² analysis of samples at atmospheric pressure,³ desorption atmospheric pressure photoionization,⁴ matrix-assisted laser desorption electrospray ionization,⁵ and desorption sonic spray ionization (DeSSI, retermed recently as EASI).⁶ Among the currently available ambient desorption techniques, EASI is perhaps

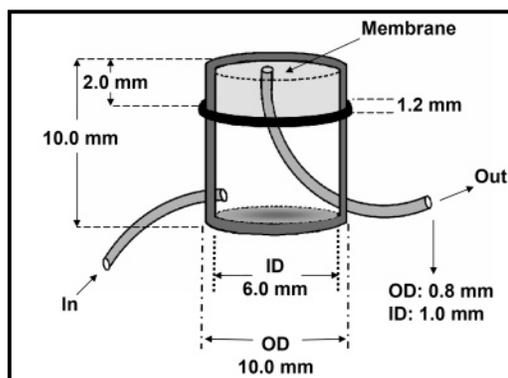


Figure 1. Details of the EASI-MIMS reservoir in which the analyte solution is circulated.

the simplest and most easily implemented since it uses no heating, no high voltages, no laser beams, no UV lights, no corona discharges, and no auxiliary gases, relying solely on the room-temperature supersonic spray to provide a dense cloud of charged (ion-carrying) droplets that produce proper analyte pickup and the gentlest⁷ ionization. EASI-MS has recently been described and has now been applied with success to several analytes and matrixes such as drug tablets,⁶ vegetable oils,⁸ perfumes,⁹ and wine.¹⁰

To the liquid surfaces of solutions, desorption techniques based on analyte pickup by solvent droplets are, however, hardly applicable due to their inherent characteristics. For solutions, therefore, a solid but permeable and flexible membrane could be used as an interface. In mass spectrometry, the use of such membrane interfaces for selective transport, trapping, and enrichment of target analytes from solutions into the high-vacuum environment of mass spectrometers has been extensively investigated.¹¹ This technique, known as membrane introduction (or

* To whom correspondence should be addressed. E-mail: eberlin@iqm.unicamp.br.

[†] State University of Campinas.

[‡] University of Helsinki.

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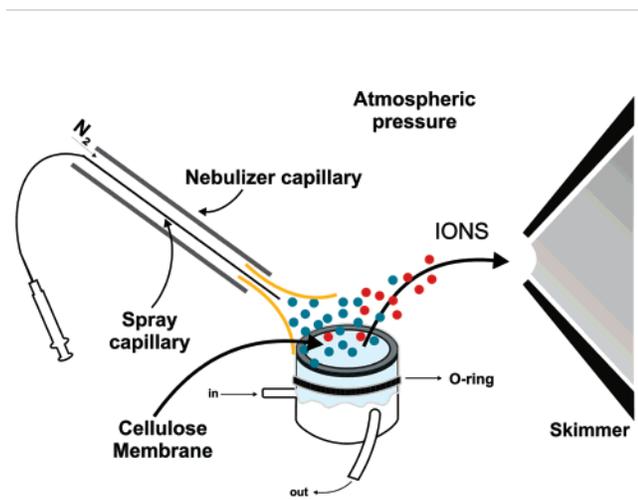
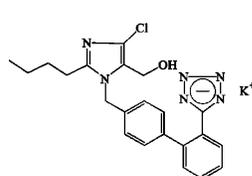


Figure 2. Schematics of the EASI-MIMS system. A supersonic spray of charged droplets bombards the surface of a cellulose membrane in direct contact with the aqueous analyte solution. Pickup by the charged droplets causes desorption/ionization of the pre-concentrated analyte that has selectively permeated from the solution to the external surface of the solid membrane.

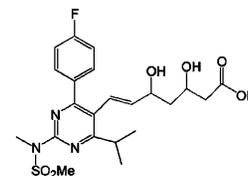
inlet or interface) mass spectrometry (MIMS)¹² and its derived techniques such as CT-MIMS,¹³ TR-MIMS,¹⁴ PAM-MS,¹⁵ TPD-MIMS¹⁶ and RP-MIMS,¹⁷ has been performed with a variety of membranes selected to optimize the transport of molecules according to their size, volatility, or polarity.

Silicone membranes (PDMS) have been extensively used with less polar analytes from aqueous solutions whereas porous membranes have been shown to allow the analysis of nonpolar and polar compounds from common solvents.^{17,18} For example, ethanol and 2,3-butanediol were measured from hexane and toluene¹⁷ using a microporous polypropylene membrane, whereas a microporous poly(vinylidene fluoride) membrane was used for monitoring of the Michael addition reaction of phenylethylamine and acrylonitrile in ethanol,^{18b} and a microporous cellulose membrane has been used to measure, for example, pyridine and 1-pentanol in toluene with a miniaturized MIMS instrument.^{18a} Dialysis membranes have also been used for aqueous extractions of amino acids, peptides, and other types of molecules of biological significance.¹⁹ In addition, since the partitioning is not significantly dependent on the chemistry of the membrane material but mainly

Chart 1. Structure, Commercial Name, Major Component, Molar Mass, Manufacturer, and Amount of Drug Per Tablet for the Set of Drug Tablets Investigated



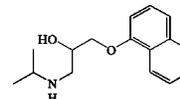
(1) Aradois - Lozartan Potassium
C₂₂H₂₂ClKN₆O = MW = 461.13
BioLAB - 25 mg



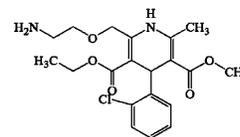
(2) Crestor - Rosuvastatin calcium
C₂₂H₂₈FN₃O₆S - MW = 481.17
AstraZeneca 10mg



(3) Ebix - Memantine Hydrochloride
C₁₂H₂₁N - MW = 179.17
Lundbeck Brasil - 10 mg



(4) Propranolol - Propranolol Hydrochloride
C₁₆H₂₁NO₂ - MW = 259.16
10 mg



(5) Norvasc - Amlodipine besylate
C₂₀H₂₅ClN₂O₅ - MW = 408.15
Pfizer - 5 mg

on the size of the pores, polar and less polar analytes are both extracted at comparable ratios.

Herein we describe the first coupling of an ambient DeSSI with MIMS (the I stands for interface). Using aqueous solutions of common drugs as a proof-of-principle example, we show that permeable and flexible solid membranes can be used as an interface to promote droplet pickup of analytes from the liquid surface of solutions with the desirable selectivity.

EXPERIMENTAL SECTION

Chemicals. Formic acid, nicotine, and HPLC-grade methanol were purchased from Merck SA (Rio de Janeiro, Brazil) and used without further purification. Deionized water was obtained from a MilliQ (Millipore, Billerica, MA) purification unit. Chart 1 summarizes information of the active drug components for the set of five commercial drug tablets selected for this study. Cellulose membranes, purchased from Viskase Co., Inc., were Membra-Cel/dialysis membranes (MD34–14) with a molecular mass cutoff of 14 000 Da and thickness of 23 μ m (0.9 mils). The drugs tablets were dissolved in water (100 mL) with no further preparation.

Mass Spectrometry. Experiments were performed on a Q-trap hybrid triple–quadrupole mass spectrometer (Applied Biosystems do Brasil, São Paulo, Brazil) using a homemade SSI source similar to that described by Cooks et al.²⁰ and mounted on a commercial nano-ESI source (Applied Biosystems do Brasil), which is described in detail elsewhere.⁴ The mass spectrometer was operated in the positive-ion mode with the following major operating parameters: flow rate of the 1:1 acidic (0.01% formic acid) water/

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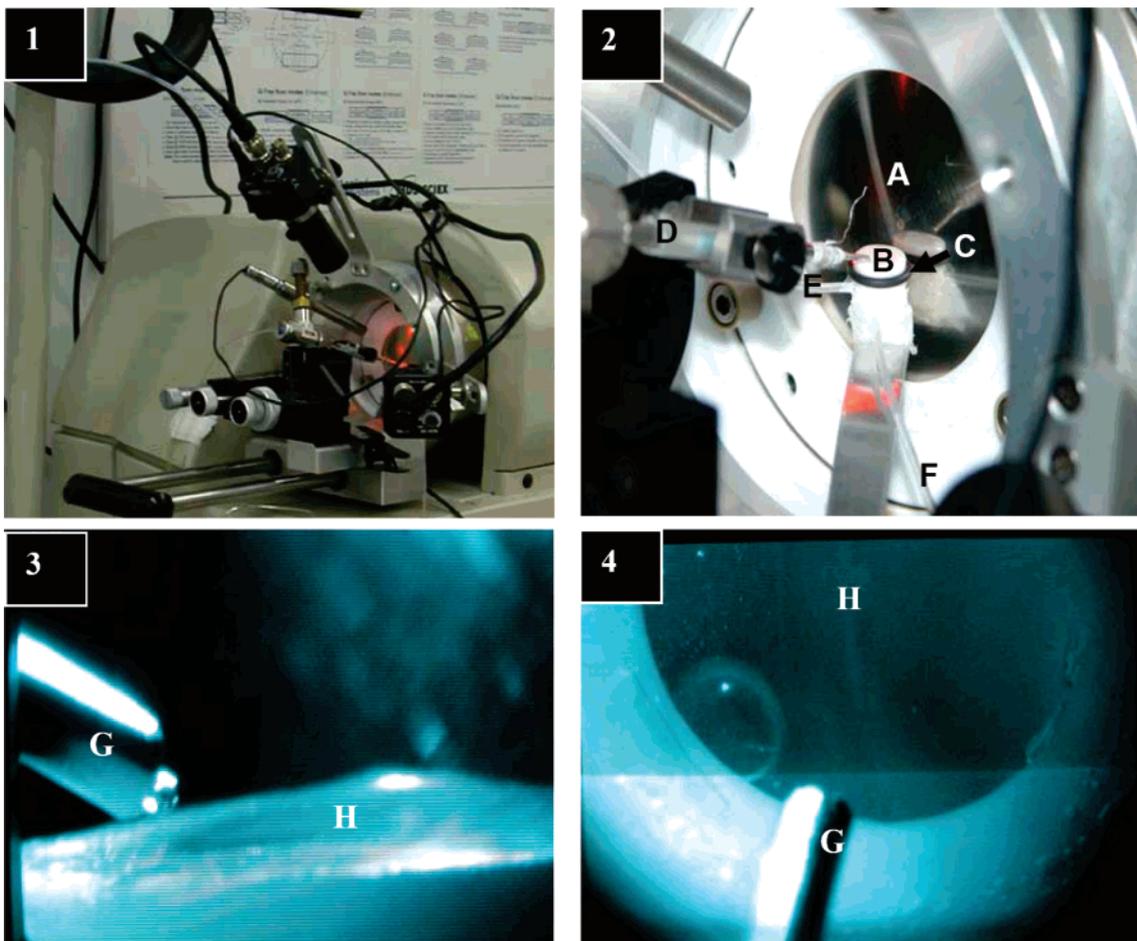


Figure 3. Pictures of the EASI-MIMS system in operation. (1) Overall view of the system, (2) closer view, and (3) side and (4) upper view of the external membrane surface being bombarded by the sonic spray charged droplets. (A) MS entrance (skimmer), (B) MIMS reservoir, (C) O-ring used to hold the cellulose membrane, (D) SSI source, (E) analyte solution entrance, (F) analyte solution exit, (G) SSI capillary, and (H) external surface of the cellulose membrane.

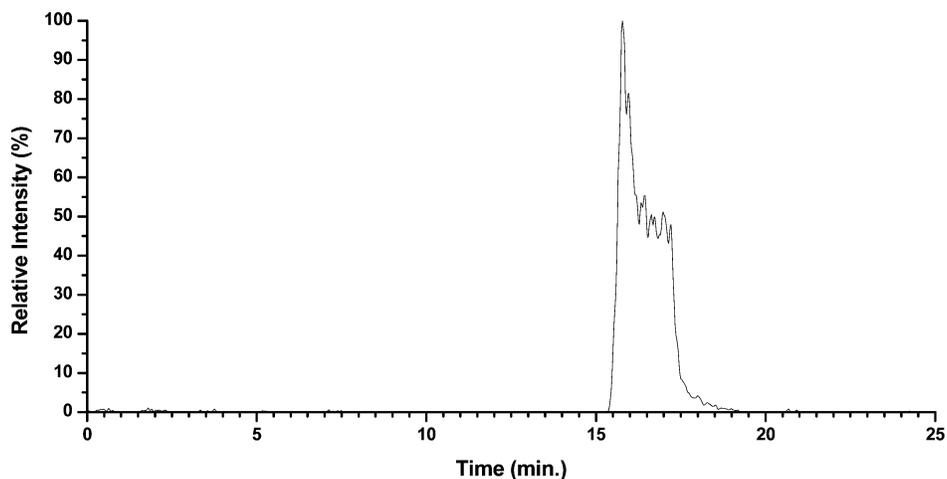


Figure 4. EASI-MIMS signal profile for a 100 ppb aqueous solution of nicotine after 15 min of trapping.

methanol solution of $20 \mu\text{L min}^{-1}$, nebulizing gas back pressure of ~ 30 bar, curtain gas pressure of 5 bar, declustering potential of 100 V, tip–membrane and tip–entrance distances of ~ 2 mm for DeSSI, and capillary–membrane entrance angle of $\sim 30^\circ$.

EASI-MIMS System. As Figure 1 shows in detail, the membrane interface is simple and was constructed using a small cylindrical Teflon container to which two silicon tubes were

connected. Note that although a planar membrane was used, tubular membranes could also be used in similar EASI-MIMS devices. The aqueous solution of the drugs (10 mL) was continuously pumped through the EASI-MIMS system with a flow rate of 20 mL min^{-1} in a closed circuit using a peristaltic pump. The cellulose membrane was placed on the top of the container and held in place using an O-ring. A 1:1 acidified water/methanol

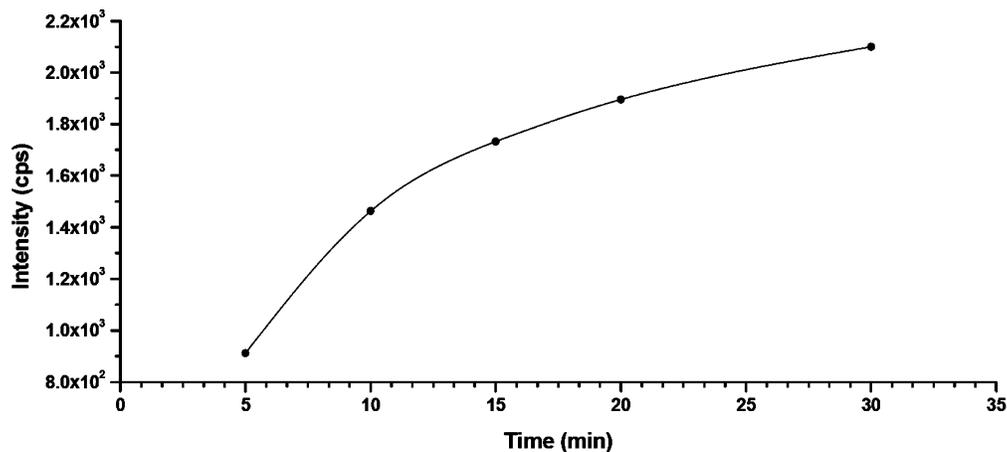


Figure 5. EASI-MIMS responses (for the protonated molecule of m/z 163) as a function of trapping time for a 100 ppb aqueous nicotine solution using a 1:1 acidic water/methanol SSI solution.

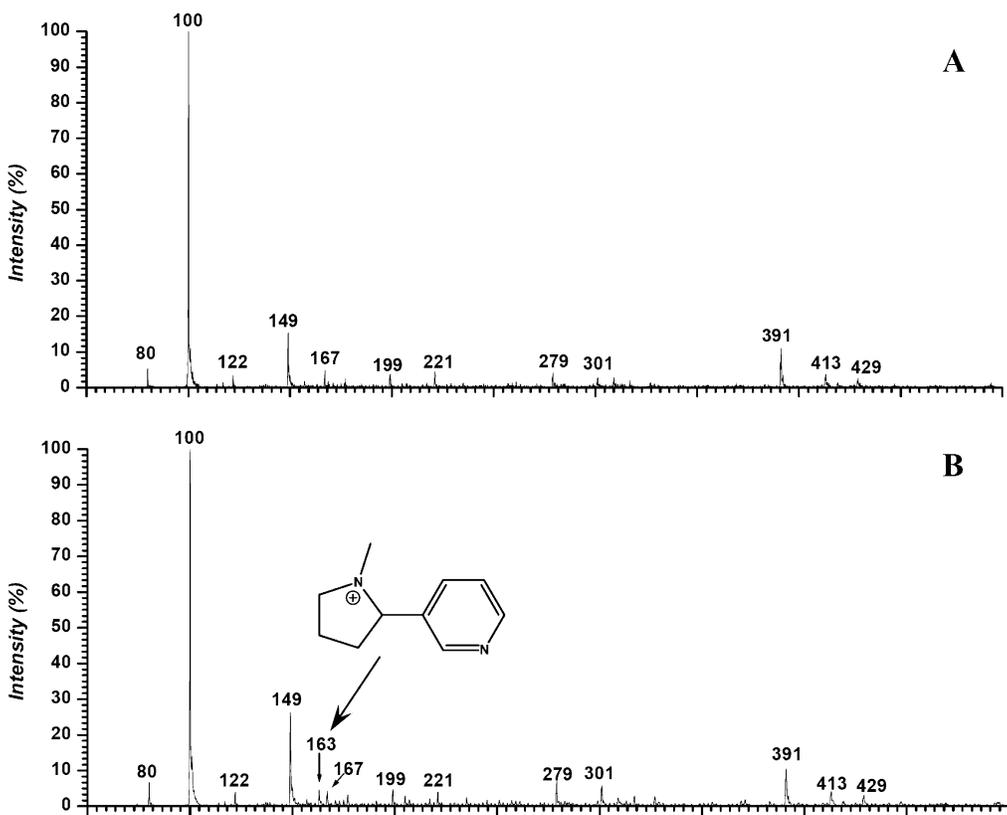


Figure 6. EASI-MIMS of (A) a blank aqueous solution and (B) a 50 ppb aqueous nicotine solution.

solution was then sonic sprayed, and the resulting charged droplets bombarded the external membrane surface at an optimum angle of $\sim 30^\circ$. This process promoted pickup and ionization of the analytes that permeate from the solution as Figure 2 shows schematically. Figure 3 shows actual pictures of the system in operation.

RESULTS AND DISCUSSIONS

Membrane Interface. To optimize the EASI-MIMS system, a 100 ppb aqueous solution of nicotine was used as a reference solution. Protonated nicotine of m/z 163 was monitored, and Figure 4 shows the corresponding signal profile for EASI-MIMS obtained after 15 min of trapping (see EASI-MIMS Trapping).

Table 1. EASI-MIMS Detection Limits after 15 min of Trapping for Aqueous Solutions of the Five Drugs Tested

drug	$[M + H]^+$ (m/z)	LOD (S/N = 3) (ppb)
memantine	180	10
crestor	482	50
propranolol	260	10
aradois	462	50
norvasc	409	20

During the time in which pumping of the acidic 1:1 water/methanol SSI solution was turned off, the analyte (from the

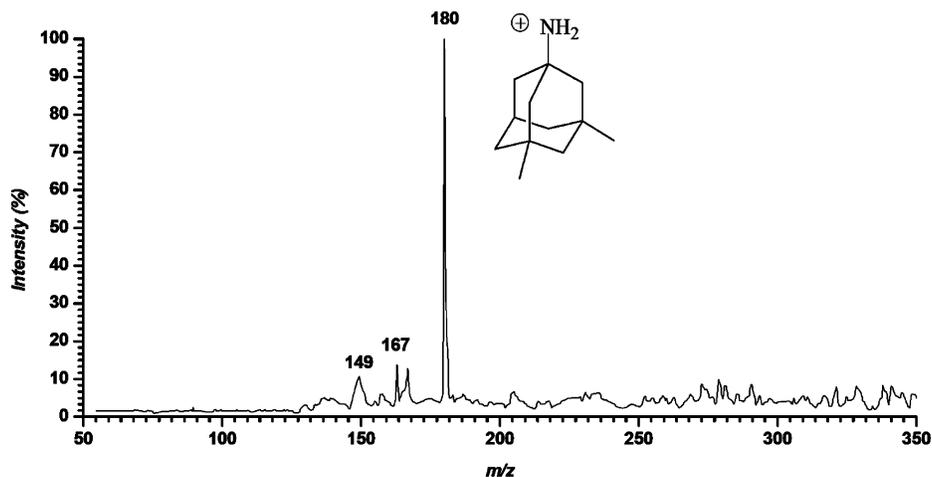


Figure 7. EASI-MIMS of a 10 ppb aqueous solution of memantine, after background subtraction.

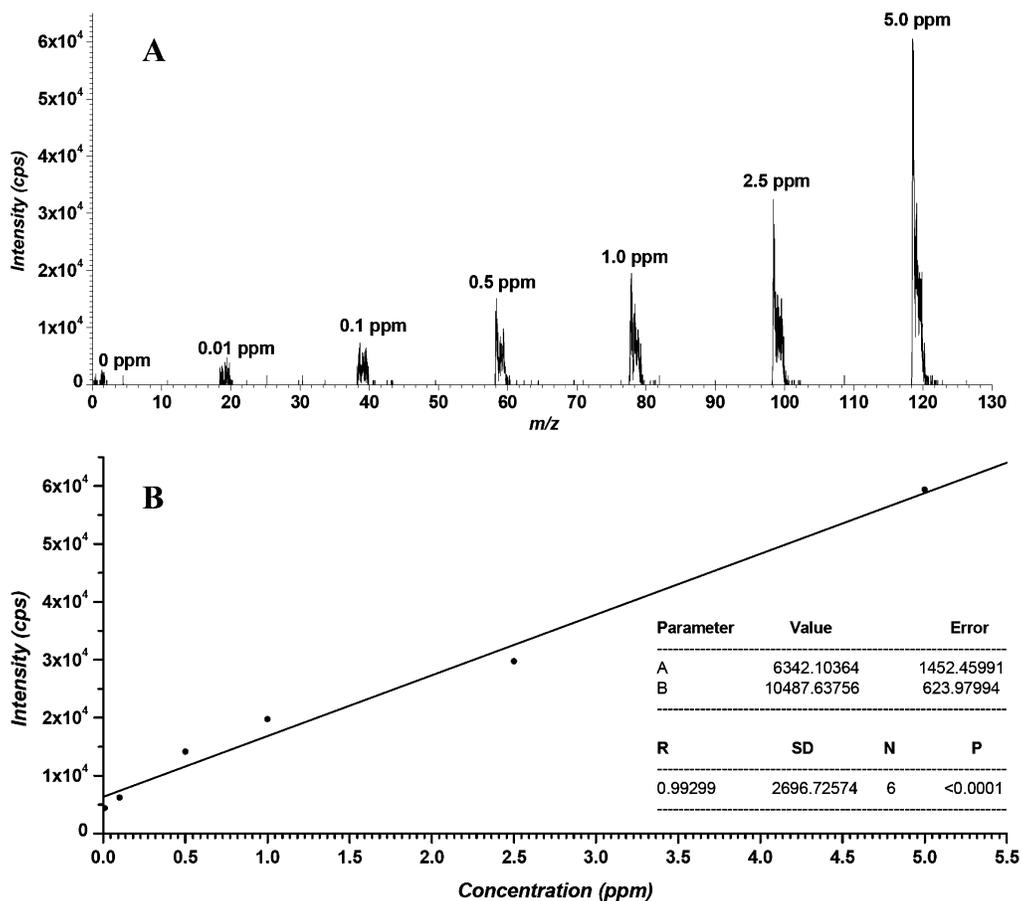


Figure 8. (A) EASI-MIMS responses using SIM of the ion of m/z 163 (protonated nicotine) as a function of solution concentration and (B) the respective analytical curve.

aqueous solution being continuously pumped through the MIMS reservoir) permeates the membrane and is deposited on its external surface. The membrane surface is continuously dried by the action of the nitrogen SSI gas flow. After the trapping period, the syringe pump is turned on with a solution flow rate of 20 mL min^{-1} . As Figure 4 shows, the sonic spray charged droplets that are now bombarding the external membrane surface rapidly pick up the analyte, causing its efficient desorption and ionization. Therefore, an intense and \sim 2-min-wide EASI-MIMS peak is detected. The spatial resolution of our DeSSI system was

measured to be \sim 10 mm, and a longer signal could be obtained by scanning the spray along the membrane surface.

EASI-MIMS Trapping. The sensitivity for continuous EASI-MIMS in our API 2000 Q-trap mass spectrometer²¹ for the test solution (100 ppb aqueous nicotine) was too low, so pulsed EASI-MIMS was applied in a trapping and release (TR-MIMS) strategy.¹⁴ Figure 5 shows the variation in signal intensity (peak area) as a function of the pulse interval (trapping time). Analyte response

(21) Sensitivity is expected to be much higher for newer versions of the Q-trap instrument or other highly sensitive mass spectrometers such as ion traps.

increased nearly linearly with trapping time up to ~10–15 min, but the signal started to level off significantly after 15 min of trapping. Although the EASI-MIMS perform may vary considerably according to the type of porous membrane and analyte used, based on the data from Figure 5, we selected 15 min as the standard trapping time in our experiments for the best speed/sensitivity ratio.

Membrane Background. Figure 6A shows a blank DeSSI-MS spectrum for pure water. Several ions are detected, probably due to trace contaminants from the cellulose membrane, from the water, or from both. This spectrum indicates that although EASI-MIMS is attained for an aqueous solution due to membrane permeation, likely both the membrane and the solvent provide background ions that may interfere with analyte detection and quantitation. The background ions are, however, relatively few and most analyte ions could be detected unambiguously at low concentrations, as Figure 6B shows for a 50 ppb aqueous solution of nicotine (m/z 163). For full scan acquisition, this minor chemical noise can be eliminated or highly minimized by background subtraction, as Figure 7 shows for a 50 ppb aqueous solution of memantine of m/z 180. For the monitoring of most known analytes, selective ion monitoring with internal standards will naturally not be influenced by the membrane/matrix noise as far as non-coincident m/z are monitored or MS/MS is used for improved selectivity.

Linearity. Figure 8A shows EASI-MIMS profiles using SIM of the ion of m/z 163 when testing for linearity in the 10 ppb–5 ppm range using a serial dilution of an aqueous solution of nicotine, whereas Figure 8B presents the respective calibration curve (peak area). Note the good linear regression coefficient ($R^2 = 0.993$), which indicates good linearity for the EASI-MIMS system.

Limits of Detection (LOD). Table 1 displays the LOD in aqueous solutions for the five drugs tested. These relatively low LOD show that relatively high sensitivity can be achieved with our EASI-MIMS system after 15 min of trapping. Note that

although the polarity of the analytes varies considerably, their LOD were low and relatively similar.

CONCLUSION

The use of membranes as interfaces for direct analysis of solution constituents via easy ambient desorption sonic-spray ionization mass spectrometry has been demonstrated. Using aqueous solutions of common drugs as a representative example, DeSSI-MS has been shown to promote efficient analyte permeation (through the membrane) and pickup (by the sonic spray charged droplets). The analytes, after permeating the membrane and being trapped and preconcentrated on the external membrane surface, were desorbed, ionized, characterized, and quantitated by mass spectrometry. Several important applications of EASI-MIMS can be envisaged. It could be used for continuous on-site monitoring of target polar and semipolar contaminants in environmental fluids such as industrial effluents. The on-line monitoring of fermentation and biotransformations by EASI-MIMS should also be beneficial since permeable membranes able to block solid particles, microorganisms, and other chemical constituents such as proteins, sugars, and bio-polymers in general could be used. These dialysis membranes can also be used to monitor hemodialysis processes or to construct very small probes, which may permit in vivo blood analysis (for pharmacokinetic studies, for instance) via the intercalation of miniature EASI-MIMS probes to veins.

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