

//// Fluid dynamics of the open port interface for high-speed nanoliter volume sampling mass spectrometry

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ABSTRACT

The open port interface (OPI) coupled to an atmospheric pressure ion source is used to capture, dilute, focus, and transport nanoliter volume sample droplets for high-speed mass spectrometric analysis. For typical application, the system has been optimized to achieve 1 Hz nanoliter volume sample transfer rates, while simultaneously diluting the sample >1000-fold to minimize sample matrix induced ionization suppression [1-2] Geometric, flow, and dispensing alterations to the system presented here demonstrate that sample transfer rates for the OPI of at least 15 Hz are possible. The fluid dynamic processes that enable sampling rates of 1 Hz and greater are examined in detail by correlating computational fluid dynamics simulations, analytic calculations, experimental data, photographic footage, and reference to the fluid dynamics literature. The resulting models and experimental results provide the rational underlying the design and tuning of the system as well as information for developing optimized analytical methods. In combination with acoustic droplet dispensing, referred to as Acoustic Ejection Mass Spectrometry (AEMS), this system can be considered to be a special case of flow injection analysis with unique features that control the peak width, symmetry, and segregation of the samples transported in a fluid while simultaneously enabling their mixing and dilution with carrier fluids. In addition, conditions are established to prevent direct contact of the sample with a surface enabling, in combination with a contact free dispenser like acoustic ejection, a dramatic reduction in sample-tosample carry-over.

INTRODUCTION

As an interface for mass spectrometry (MS), the Open Port Interface (OPI) captures nanoliter volume sample droplets and transports them in a fluid to pneumatically assisted electrospray ionization (ESI). The continuous liquid stream is drawn into the ion source by the Venturi effect created with a high velocity pneumatic nebulizer of the electrospray ionization. The OPI has been described as the interface used to transfer acoustically dispensed samples from microtiter plates at high throughputs for drug discovery applications. This technique has been termed Acoustic Ejection Mass Spectrometry (AEMS) [1-2].

This study focuses on the fluid dynamics of nanoliter volume droplet capture, dilution, and transport within the OPI and their implications on the design, tuning, and development of high-speed analytical methods. The system can be considered to be a special case of flow injection analysis (FIA) with unique features that control both dispersion and segregation of captured liquid droplets while simultaneously diluting the sample in excess of 1000 fold to minimize ionization suppression.

Computational fluid dynamics (CFD), analytical calculations, photography, empirical experimentation, and reference to the fluid dynamics literature are used to elucidate the underlying fluid flow physics controlling nanoliter volume sample capture, focusing, segregation, dilution, and transport in the OPI. Dispense rates of 1 Hz are demonstrated and 15 Hz shown to be feasible. In addition, the understanding garnered from this study provides a rational basis for the optimization of OPI parameters and additional insights into opportunities for future performance improvements.

MATERIALS AND METHODS

Reagents and materials:

Dextromethorphan was purchased from Cerilliant (Round Rock, TX) and the HPLC grade methanol and acetonitrile were bought from Caledon Laboratory Chemicals (Georgetown, ON, Canada). Analytical Standard DEET was purchased from Sigma Aldrich Gmbh Laborchemikalien. Deionized water (18 MΩ) was produced inhouse using a Millipore (Billerica, MA) Integral 10 water purification system. The CO2 plasma treated microplates were purchased from Aurora Microplates. Prior to analysis the microplates loaded with sample solutions were centrifuged at 3000 RPM for 3 minutes to remove gas bubbles and provide a constant meniscus shape. Residual static charge on the surface was removed with a bath of ionized air in the ATS instrument. A well plate density of 384 was used in this study but this approach is compatible with 96 and 1536 well plates.

The acoustic dispenser was a modified ATS Gen 4+ system (EDC Biosystems, Fremont, CA) as described previously [1,3]. The time from the start of the acoustic energy pulse for droplet dispense to mass spectral peak detection was used to determine the velocity of the sample in the transport tube. Drop flight to the OPI and the ion transport within the mass spectrometer accounted for ~3 msec of this measurement time typically in the 2-5 sec range.

OPI-MS:

A high-speed OPI used a pneumatic dispenser and variable length transport tubes. The OPI in the AEMS system was the same as reported previously. A SCIEX Triple Quad 6500+ system with an OptiFlow ion source was used.

CFD modeling:

The solver interMixingFoam from OpenFOAM 4.x was used to simulate the droplet colliding and mixing with the fluid in the sample end of the OPI. Each simulation was discretized with about 4 million hexahedral cells and solved using 64 cores on one shared memory compute node. The time stepping scheme was explicit Euler. Gradient, Laplacian and interpolation schemes were all linear. Advection of velocity and alpha was solved using the linear limited scheme. Surface normal gradients for surface tension calculations were corrected. Each simulation ran for 96 hours with the 0.01-sec physical simulated time interval. The steady state calculations used to re-create fluid surface morphology and fluid velocities (no droplet) each took about 12 hours on 64 cores to achieve steady state.



RESULTS

Figure 2 shows ramping the inlet flowrate over the different OPI capture conditions. In super-critical condition, the fluid surface deeply funneled, and an air core extended down into the transport tube, where the signal is unstable, and the signal peaks are widened. For the transition and dome conditions where the inlet flow exceeds the pumping capacity of the venturi pump, the peaks are widened and merged into a near continuous signal. The fluid surface in the critical condition is cone shaped, where the ion current is stable, and the peaks are at their narrowest.







Acoustic dispenser:



Figure 1. AEMS system. A. **OPI** capture port oriented downward. A'. Drawing of

critical condition surface in "A". B. OPI with a 50 cm transport tube. C. OPI venturi pump/ESI nebulizer. C'. Sonic expansion creating pressure drop. D. Fluid delivery pump. E. Acoustic dispensing upward against

Figure 2. Chronograms of three conditions of flow balance including photographs & cross-sectional drawing of surface with velocity vectors. 30 samples dispensed acoustically at 1 Hz. 50 cm transfer tube. A. Supercritical Condition illustrating air penetration & its effect. B. Critical Condition with expanded x-axis below. C. Dome Condition illustrating stagnation zones & the effect on ion current.



Figure 3. A. Super critical condition fluid velocity profiles from CFD simulation. Methanol flow rate = 200 µL/min with 50 cm transfer tube. B. Dome condition fluid velocity profiles from CFD simulation. Methanol flow rate = 550µL/min with 50 cm transfer tube. C. Critical condition CFD generated fluid velocity profiles. 5 nL droplet drawn to scale is superimposed on the simulation for perspective. Methanol flow rate = 500 μ L/min with 50 cm transfer tube.

The accelerating fluid and the viscous shear pressure encountered cause the sample to stretch as it enters and traverses the initial length of the transport tube. This process of droplet stretching is attenuated by the existence of additional low velocity regions at the apex of the cone which is adjacent to a fast-moving fluid. The sample is observed to be feeding into the transfer tube with the trailing end in a lower velocity fluid than the leading front. This caused the dilution in 800 msec resulting in an ~10-20 cm long sample plug formed in the initial length and center of the transport tube.



Shortening the tubing length increases the fluid linear velocity proportional to the reduction. Different transfer tube lengths were tested using the port described in Fig. 1. A pneumatically driven nanoliter dispenser designed for 3D printing of biological materials and matrices delivered the droplets from a bulk reservoir in a syringe barrel and provided the mechanical flexibility to enable tube length reduction compared to the acoustic ejection technology at hand.

A 4.8 cm transport tube (Figure 5) delivering low viscosity acetonitrile increased the critical condition flow rate to 2.5 mL/min. A sampling rate of 10 Hz is shown in Fig. 6 with peak widths at baseline of 68 ms indicating that at 15 Hz peaks would remain baseline separated at 1% peak height enabling an assay dynamic range, or abundance sensitivity [1], of at least 100.



Figure 4. A. Photograph of stretching and centerline focusing of a 5 nL dye containing droplet. B-D. Time sequenced images from the CFD analysis of droplet capture and dilution over 6.26 msec. Only the first 10 msec after impact is simulated due to the computer overhead required to perform these calculations.



Figure 5. High-throughput setup for the data collected in Figure 6.



Figure 6. Chronogram at 10 Hz demonstrating feasibility of 15 Hz based on peak separation at baseline (1% peak height). 4.8 cm transfer tube at 2.5 mL/min acetonitrile.

CONCLUSIONS

The OPI interface for nanoliter droplet sample introduction into an ESI source is a form of high-speed flow injection analysis with unique features. Physical models of the fluid flows are elucidated to describe the mechanisms by which samples are captured, diluted, focused, and trans-ported in the fluid stream. The physical models are based on correlating information from CFD simulations, photography, analytic calculations, experimental data, and reference to the fluid dynamic literature.

Four fluid flow conditions that have implications on performance are described in terms of the fluid dynamics that define them. Conditions where air entrainment or eddy currents dominate result in poor throughput, peak shape, and ion current stability. Under conditions where the proper balance between inlet and outlet flows is established the fluid velocity vectors are positioned to rapidly clear the port and dilute the sample greater than 1000-fold with the accelerating fluid. A focusing of the sample onto the centerline of the transport tube occurs in the same region as a result of the orientation of the fluid flow vectors. This improves peak symmetry by reducing the extent of Poiseuille flow through the transport tube. Diffusion of the sample to the outer walls before exiting into the ion source completes the dilution of the stretched sample.

The transport fluid flow rate has a large influence over the sampling speed and is limited by various sources of resistance to flow. A flow choking effect caused by the fluid free surface is identified as one source. The tubing geometry and fluid viscosity are additional parameters that determine the maximum speed attainable from the pressure drop created by the venturi pump. Modifications and tuning of these parameters demonstrate that a sampling speed of at least 15 Hz is possible with the OPI interface. The speeds attainable from a variety of common solvents with varying viscosity are also provided.

The inlet and outlet fluids to the OPI are comprised of fluids that differ by the amount of air entrained in the liquid and are delivered by separate pumps. A critical balance of these flows is required to simultaneously achieve sample segregation and dilution.

REFERENCES

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