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Droplet Based Sampling of RNA Hydrolysates by Induction Based Fluidics

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Overview

The goal of this study was to couple an inductive charging device to a liquid chromatography separation with a focus of lowering the LOD for standard RNA nucleoside analysis. As such, a synthetic test mix comprised of cytidine, uridine, 5-methylcytidine, adenosine and 2'-O-methyladenosine were separated by means of capillary chromatography and delivered into the mass spectrometer by using a modified inductive charging source powered by a modified inductor coupled to a digital programmed energy and polarity pulsed DC source[1].

Introduction

Post-transcriptional chemical covalent modification of adenosine, guanosine, uridine and cytidine occurs frequently in all types of ribonucleic acids (RNAs). In ribosomal RNA (rRNA) and transfer RNA (tRNA) these modifications make important contributions to RNA structure and stability and to the accuracy and efficiency of protein translation. These modifications can be present at very low levels and their analysis can be challenging. This work builds on previous work where the utility of Inductive Based Fluidics (IBF) as a sample introduction method is examined while coupled to an LC platform. Because IBF creates inductively charged droplets instead of an electrospray, theoretically, a droplet sampling method would allow for greater sensitivity as more sample would enter the mass spectrometer.

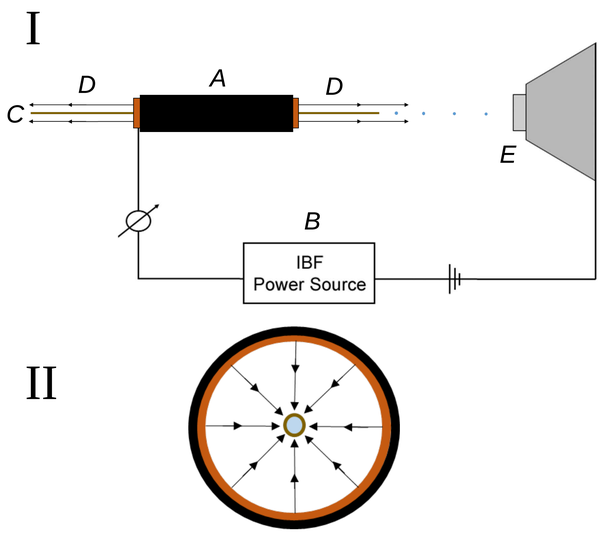

Figure 1. Schematic of inductor employed in this work, inspired by R.W. Kiser[2]
I A: Insulated copper tube (90 x 6 mm) B: Inductive charging device C: Capillary tubing (360 X 50 µm) with flow from column D: Field lines E: Inlet to mass spectrometer.
II Forward view of IBF charging tube showing nexus of field lines onto the sampling capillary

Methods

An equimolar RNA hydrolysate mixture was separated on a porous graphitic carbon packed capillary column inserted into an in-house inductive charging tube with capillary positioned 2-4 mm from inlet orifice. Mass spectra were recorded in positive polarity on a Thermo Fisher LTQ-XL. A capillary temperature of 275 °C, spray voltage of 0 kV, capillary voltage of 0 kV, and tube lens at 0 kV. IBF device was set to ~ 2000V and pulsed + and - with 2 s intervals over a 40 min acquisition. Data acquisition was through the Thermo Fisher Xcalibur software.

Results and Discussion

Five RNA nucleoside standards, cytidine, uridine, 5-methyluridine, adenosine, and 2'-O-methyladenosine were separated and sampled using the IBF device. Extracted ion chromatograms of the analytes are shown in Figure 2.

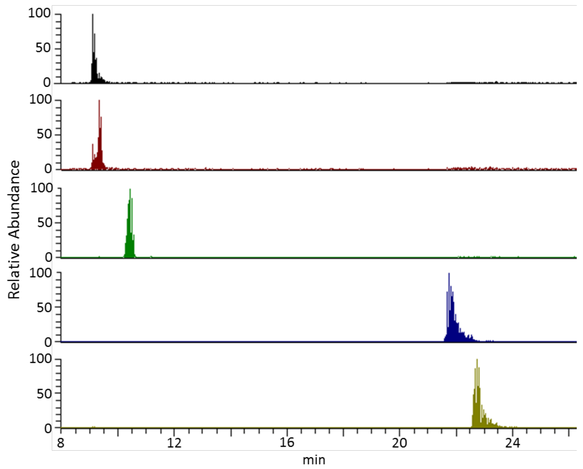

Figure 2. Extracted ion chromatograms of nucleosides cytidine, uridine, 5-methyluridine, adenosine, and 2'-O-methyladenosine separated on a PGC capillary column and introduced into the mass spectrometer by inductive charging.

Droplets were delivered with a 2 s interval over a total run time of 40 min. The total ion chromatogram (TIC) showed steady reproducible droplet peaks throughout the gradient. Each peak in the TIC corresponds to a single droplet delivered via IBF, Figure 3.

XICs of individual nucleosides were generated, with a signal response generated over a single droplet peak in the analyte elution or across the entire set of droplet peaks generated from each analyte. The mass spectrum obtained when summing across the entire acquisition window illustrates one advantage of pulsed operation wherein the background is significantly reduced as illustrated in Figure 4.

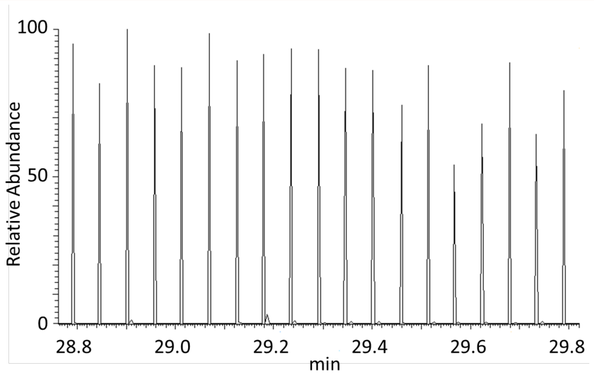

Figure 3. IBF-LC-MS/MS data of droplet introduction over a one minute acquisition window.

The intervals between droplet arrival in the mass analyzer are characterized by no background, which can be reflected in the summed mass spectral data. More Importantly, the ion abundances present within a single droplet are similar to the integrated peak values as previously shown by Groenwold, et al.[3]

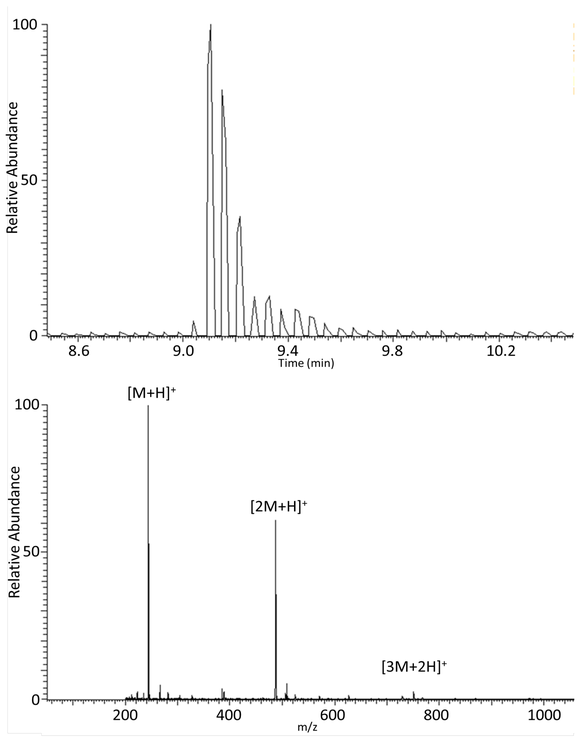

Figure 4. IBF-MS data of the nucleoside cytidine introduced dropwise by inductive charging. (a) Total ion chromatogram showing droplet introduction. (b) Mass spectrum of cytidine obtained by averaging over one peak in the acquisition window.

Droplet desolvation may be more efficient than nESI. This could limit sampling bias for mixtures if ion generation is influenced more by the kinetics of desolvation rather than the thermodynamic partitioning of the analytes with different hydrophobicities at the droplet surface. Figure 5 shows XICs of the equimolar nucleoside mixture with the relative abundances listed. This data aligns with previous work[4] suggesting that kinetics may better represent ion generation with droplet sampling. More experiments are planned to strengthen this argument

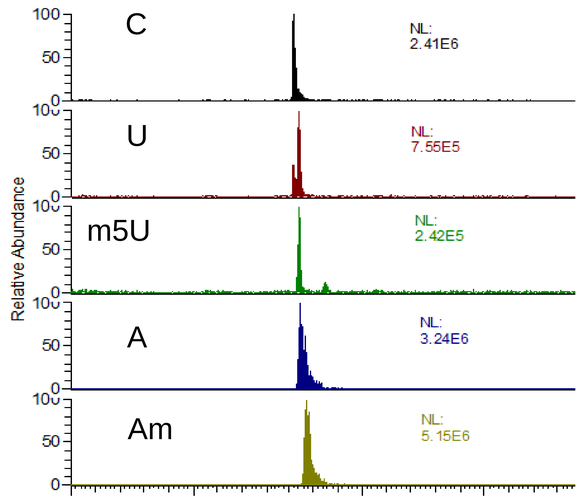

Figure 5. Mass spectra of nucleosides (5 ng/µl), cytidine, uridine, 5-methyluridine, adenosine, and 2'-O-methyladenosine. Droplet introduction could minimizes the sampling bias related to nucleobase hydrophobicity

Conclusions

A programmable IBF droplet source appears suited for nucleoside UPLC sample introduction and mass spectrometric analysis. Preliminary results show this droplet based approach is equivalent to or may exceed nESI. Work to determine LOD's and more is continuing.

References

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Acknowledgments

The presenting author would like to thank the members of the Limbach Research Group for their assistance. Financial support of this work was provided by the National Science Foundation (CHE1212625). The nanoLiter Programmable Wave device was acquired under license from nanoLiter LLC