MALDI-TOF-MS Sample Preparation for Synthetic Polymers via Nanoliter Induction Based Fluidic Deposition

UNIVERSITY OF SOUTH FLORIDA

Brent Hilker[1]; Kevin Clifford[1]; Julie Harmon[1], Ted Gauthier[1], Andrew Sauter Jr.[2]; Andrew Sauter III[2]

[1]*University of South Florida, Tampa, FL;* [2]*Nanoliter, LLC, Henderson, NV*

Overview

Purpose

- Evaluate Novel *Nanoliter Induction Based Fluidics* (IBF) versus Micropipette and Syringe Pump depositions for MALDI using dried droplet technique.
- Characterize selected polymers in each deposition method by:
  - (M_n), (M_w), (PD), P_{SP}, (S/N), Rs
  - Deposition Homogeneity

Methods

- Polystyrene (PS), poly(methyl methacrylate) (PMMA), poly(ethylene glycol) (PEG) were MALDI analyzed:
  - With M_n & M_w ranging 2500-100,000 dA
- Homogeneity of deposition is classified using LEICA DMRX Cross polarization microscope

Results

- Nanoliter IBF depositions
  - Show increased S/N, Intensities, P_{SP}
  - More Accurate M_n, M_w, and PD values
- Greater homogeneity (sweet/hot-spot) of sample deposition

Introduction

The nanoliter wave, *picture 1*, makes use of induction based fluids (IBF) which is a novel technology that dispenses *exact* nanoliter volumes of solutions by electrokinetic means *(1)*. IBF is 'green' in its capacity to dispense small volumes, significantly reducing waste of highly toxic solvents and chemicals. Coupled with MALDI mass spectrometry using Ionic Liquid Matrices (ILM) IBF has been shown to improve results by making solutions homogeneous, resulting in greater signal-to-noise ratios *(2)*.

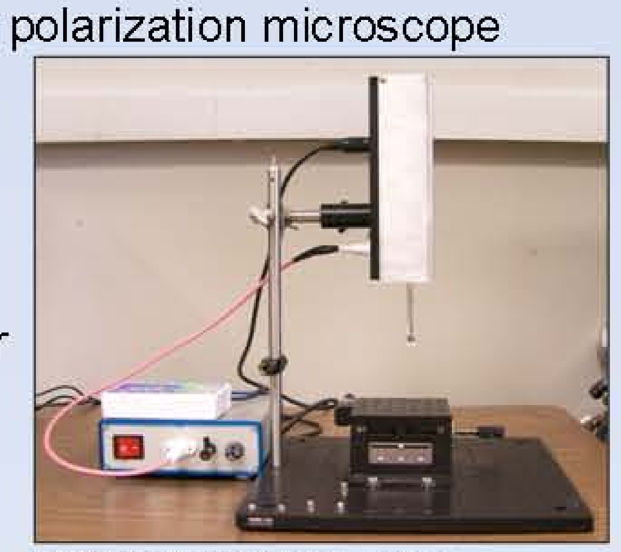
The induced charge on the droplet to be dispensed via IBF is correlated to surface area *(3)* and is 'soft' showing no undesired electrochemistry on the analytes within the droplet *(1-4)*. MALDI allows the rapid determination of: modal (M_p), molecular weight average (M_n), molecular number average (M_n), polydispersity (PD), and polymer spread (P_{sp}) *(5)*.

$$M_n = \frac{\sum N_x M_x}{\sum N_x} \quad M_w = \frac{\sum N_x M_x^2}{\sum N_x M_x} \quad PD = \frac{M_w}{M_n} \quad P_{sp} = \frac{\left|\Delta w_{\frac{1}{2}}\right|}{MM_{rep\ unit}}$$

A comparison is made of IBF deposition method versus the micropipette and syringe pump which are all methods for solid matrix dried droplet (DD) depositions. DD depositions are known to be heterogeneous and this fact afflicts the reproducibility of the MALDI signal results

It is well known that the more homogeneous samples exhibit improved reproducibility *(6)*. Homogeneity was visually observed through the use of a LEICA DMRX cross polarization microscope Cross polarization images were obtained for sample DD depositions.

A wide mass range of polymers and various MALDI recipe matrices are employed to evaluate the scope of use for the Nanoliter IBF device. These diverse variables subject this novel deposition method to 'real world' scenarios that will be encountered in laboratories.



Picture 1. Nanoliter IBF device

Methods

Poly(ethylene glycol) (PEG), poly(methyl methacrylate) (PMMA), and polystyrene (PS) are utilized in different recipes and molecular weights, **figure 1**, for (DD) deposition analysis. MALDI (DD) recipes were mixed in a [1:10:1] ratio, [Polymer: Matrix: Ion source] using tetrahydrofuran (THF) as solvent. Deposition volumes ranging from 500nl – 50nL are spotted for comparison using micropipette and nanoliter IBF device. Polymer Spectra for PEG and PS are obtained using a Voyager DE STR MALDI-TOF in linear mode. M_n, M_w, PD, P_{sp}, signal to noise (S/N) at M_p, resolution (Rs)are determined for PEG and PS for these MALDI spectra run on Voyager. A Brucker Autoflex MALDI-TOF in linear mode is used to determine S/N at M_p for PMMA

(PS-TPB 2)
5mg/mL Polystyrene (M_n 2,300 M_w 2514)
45mg/mL 1,1,4,4 Tetraphenyl-1,3 butadiene (TPB)
5mg/mL AgTFA

(PEG 5)
5mg/mL Poly (ethylene glycol)
40mg/mL Dithranol
5mg/mL NaTFA

(PS-TPB 6)
5mg/mL Polystyrene (PS) (M_n 5320)
45mg/mL 1,1,4,4 Tetraphenyl-1,3 butadiene
5mg/mL AgTFA

(PMMA 10,600)
5mg/mL Polymethylmethacrylate (PMMA) (M_n 10,600)
40mg/mL 2,5-Dihydroxy benzoic acid (DHB)
5mg/mL NaTFA

(PS-TPB 99)
5mg/mL Polystyrene(PS) (M_n-92,600 M_w 95,050 M_p 94,400)
45mg/mL 1,1,4,4 Tetraphenyl-1,3 butadiene
5mg/mL AgTFA

Figure 1. Polymer recipes

Results

(PSTPB 2)	M_N	M_W	PD	P_{SP}	(S/N) M_p	Rs @ M_p	Laser Shots	Intensity M_p
IBF 500nL	2361	2599	1.101	16.96	2723	307	750	1.80E+04
IBF 250nL	2258	2545	1.127	18.96	3186	320	750	4.90E+04
IBF 100nL	2299	2564	1.115	18.94	3864.6	331	750	4.80E+04
MP 500nL	2415	2640	1.093	15.95	2065	321	750	1.60E+04
MP 250nL	2338	2594	1.109	15.98	1820	257	750	9.10E+03

Table 1. PS-TPB 2 data

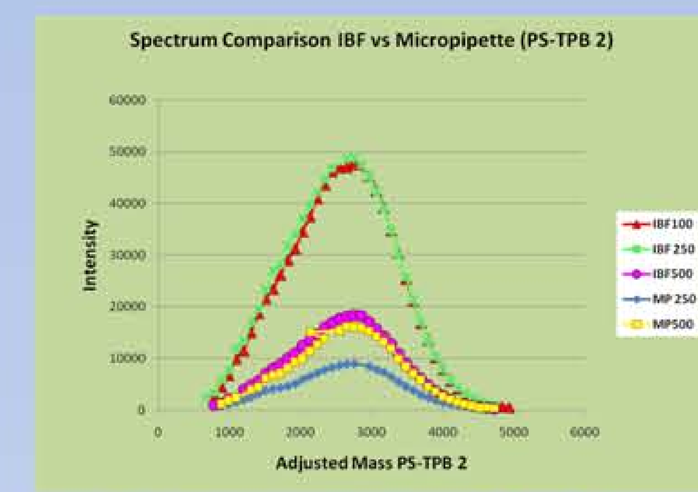


Figure 1a. Intensity comparison for PS-TPB-2

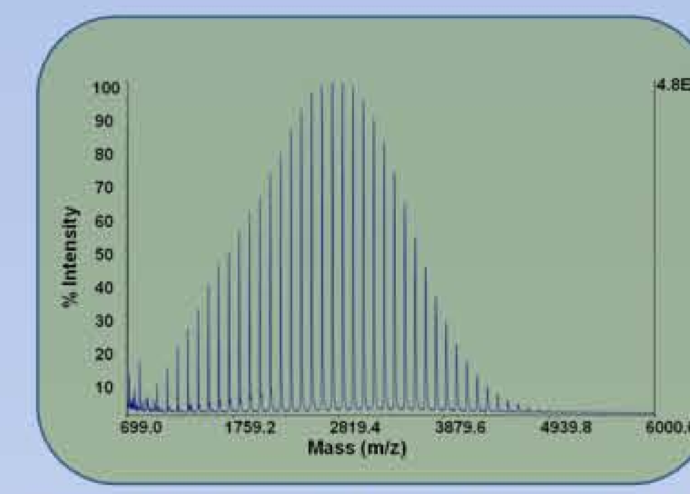


Figure 1b IBF 100nL MALDI Spectra (Raw signal) PS-TPB-2

(PS-TPB 6K)	M_N	M_W	PD	P_{SP}	(S/N) M_p	Rs @ M_p	Laser Shots	Intensity M_p
IBF 250nL	6587	6733	1.0222	23	147.5	447	750	6101
IBF 150nL	6510	6654	1.0222	22.07	111	392	750	4280
IBF 100nL	6501	6644	1.0219	20.01	126.6	546	750	3595
IBF 50nL	6409	6572	1.0255	19.03	48.3	460	500	1206
MP 500nL	6517	6650	1.0205	19	84.5	852	750	1142
MP 250nL	6466	6598	1.0204	20	87.2	847	750	1512

Table 2. (PS-TPB 6K) data

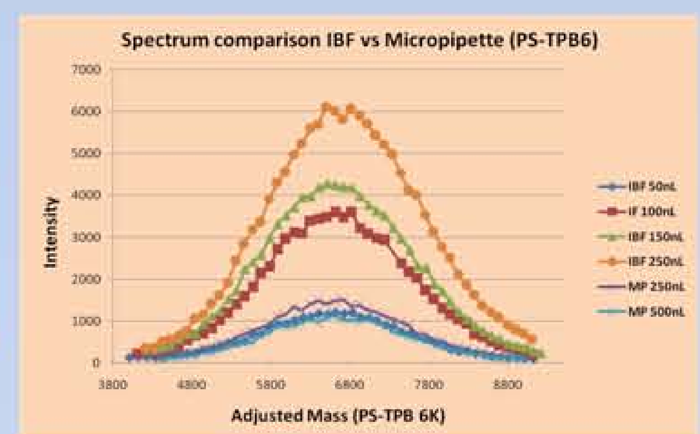


Figure 2a. Intensity comparison for PS-TPB-2

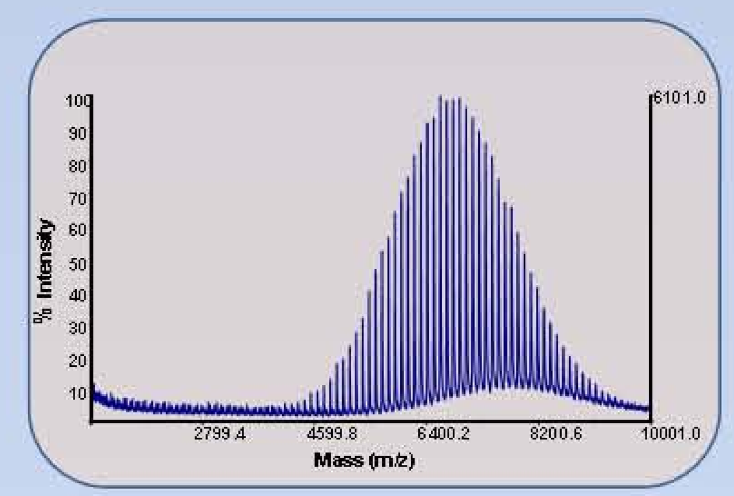


Figure 2b IBF 250nL MALDI Spectra (Raw Signal) PS-TPB-6

(PEG 5)	M_N	M_W	PD	P_{SP}	(S/N) M_p	Rs @ M_p	Laser shots	Intensity M_p
IBF 100nL	5376	5419	1.008	32	30.1	322	750	1788
IBF 250nL	5375	5418	1.008	31	28.3	330	750	1631.5
IBF 500nL	5378	5418	1.007	26	95	14,548	750	3175
SP 100nL	5069	5126	1.011	38	12.4	740	750	1529
MP 250nL	5174	5216	1.008	35	18.6	600	750	1381
MP 500nL	5099	5164	1.013	41	12.5	1048	750	1192

Table 3. (PEG 5) data

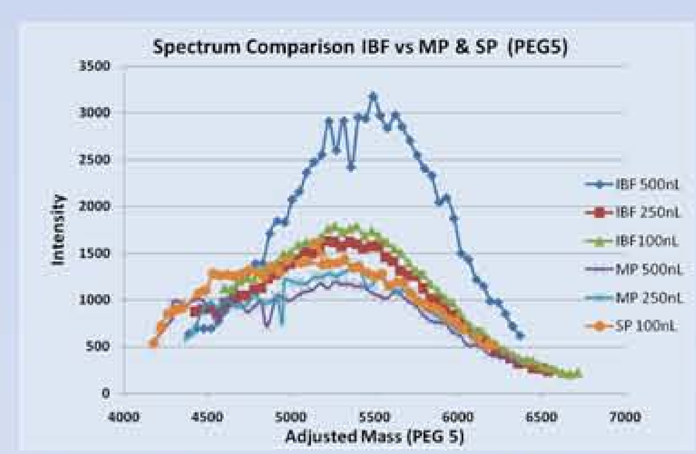


Figure 3a. Intensity comparison for PS-TPB-2

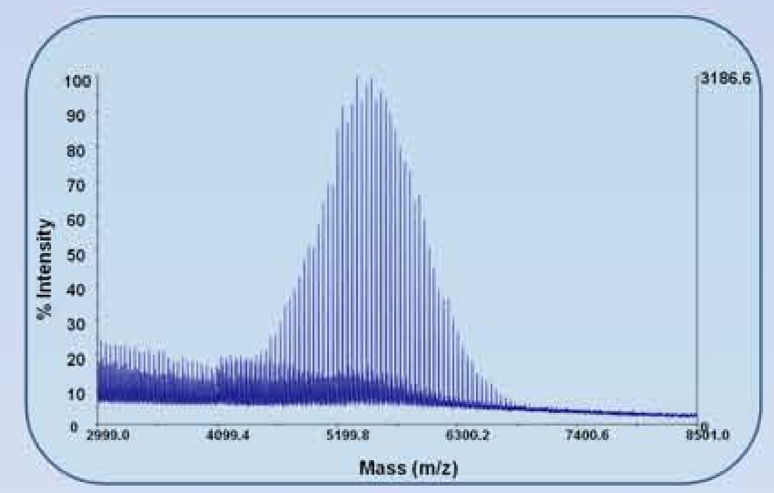


Figure 3b IBF 500nL MALDI Spectra (Raw Signal) (PEG 5)

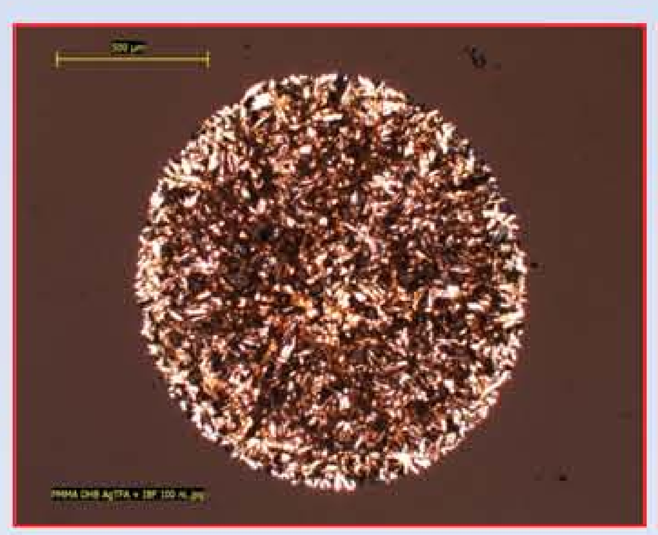


Image 1a PMMA cross polar IBF 100nL

Image 1B PMMA cross polar MP 500nL

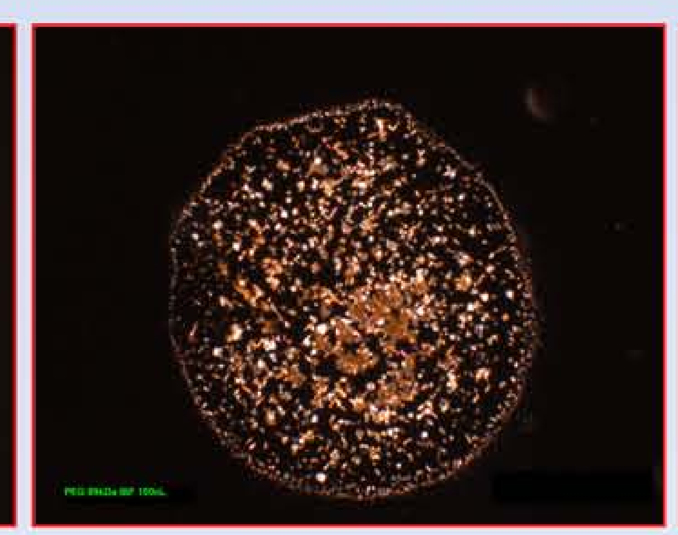


Image 1c PS-TPB 99 cross polar IBF 100nL

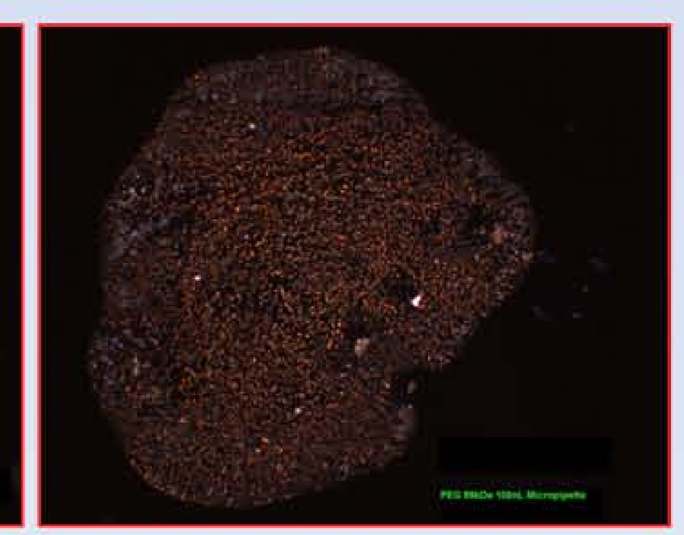


Image 1d PS-TPB-99 cross polar MP 100nL

Results (cont.)

PMMA 10.6 kDa	(S/N) M_p	Intensity M_p
IBF 500nL	12	1960
IBF 250nL	11.8	3450
MP 500nL	5	1260
MP 250nL	5.8	1598

Table 4. (PMMA) data

PS 99	S/N M_p	Intensity M_p	Rs @ M_p	P_{SP}
IBF 100nL	26.1	89	67,831	77
MP 100nL	9	39	55,214	81

Table 5. PS 99 Data

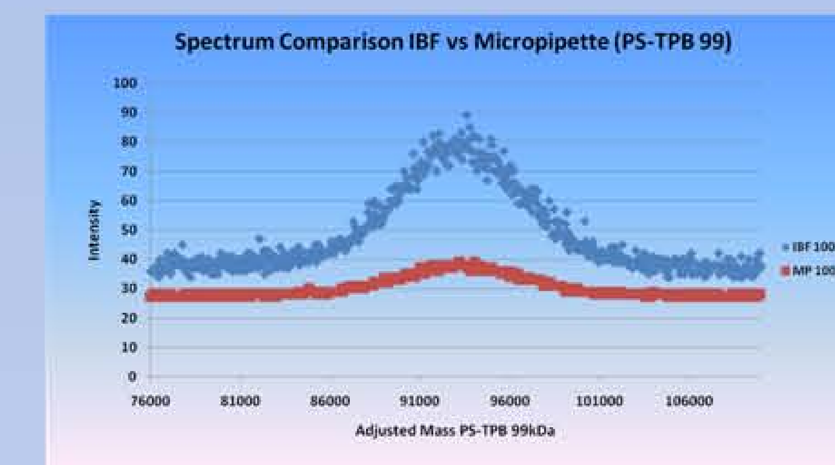


Figure 4. Intensity comparison for PPS-TPB- 99)

Conclusion

PS-TPB 2, table 1, exhibits S/N, Resolution (Rs), and intensity for decreasing volumes of IBF depositions. Table 2, shows that IBF 250nL is the optimum deposition size for PS-TPB 6 yielding the greatest S/N, Psp, and intensity. PEG 5 shows IBF 500nL depositions exhibit the greatest improvement in all categories of measurement, table 3. PMMA exhibits an increase in S/N and intensity at Mp, table 4. Table 5 shows an increase in S/N, intensity, Psp, and resolution for PS-TPB 99.

References

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Acknowledgements

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