INVESTIGATIONS ON THE EFFECTS OF SURFACTANTS IN MICROFLUIDIC SEPARATIONS AND ASSAYS

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Professor Robert Corcoran

Outline

- Reasons for this project
- Background of research
- Outline of research phases
- Phase 1, Methods and Results
- Phase 2, Methods and Results
- Conclusion

Why is This Project Necessary?

- Non-Specific Binding (NSB) prevention
  - Binding of proteins, enzymes or other biological molecules to charged glass surfaces
  - Detection of these can lead to false positives

Where it started

- Hong Kong Group, Gong & Ho
  - Testing effects of zwitterionic surfactants on the separation of proteins
- Use of surfactant caused an increase in peak sharpness during protein separation

Gong and Ho Results

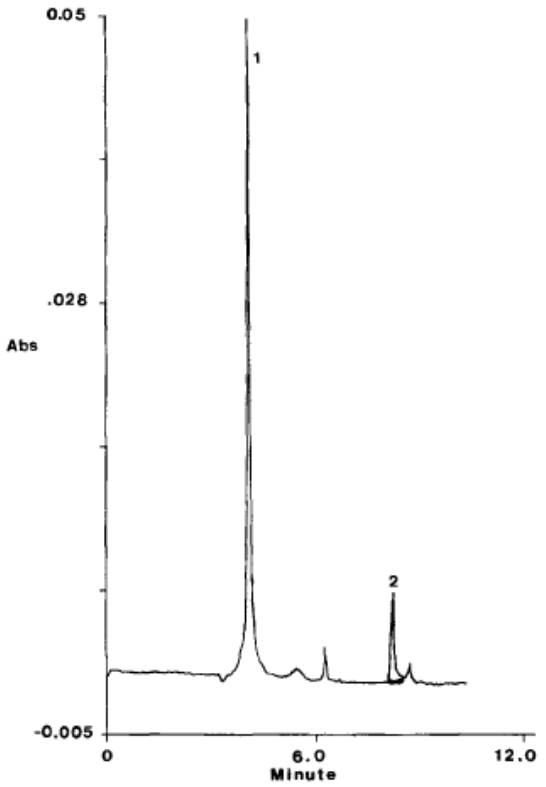


Figure 1. Electrophoretic separation of proteins with 100 mM PBS, pH 9, containing 5 mM SB3–12. Running voltage: 10 kV; 1, myoglobin; 2, lysozyme.

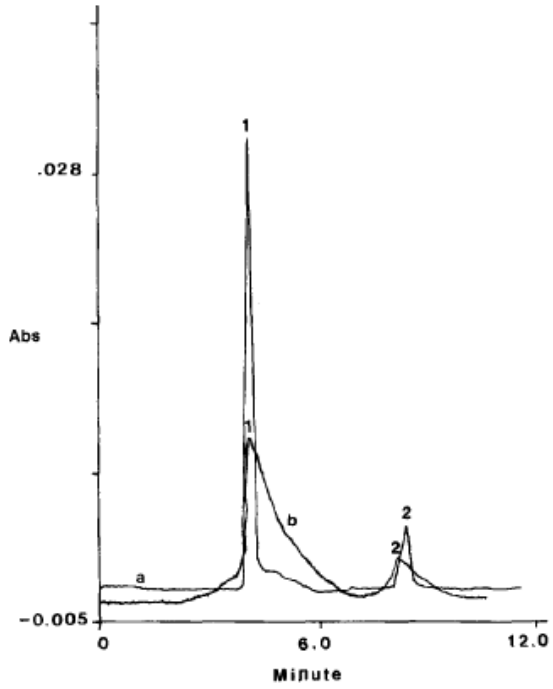
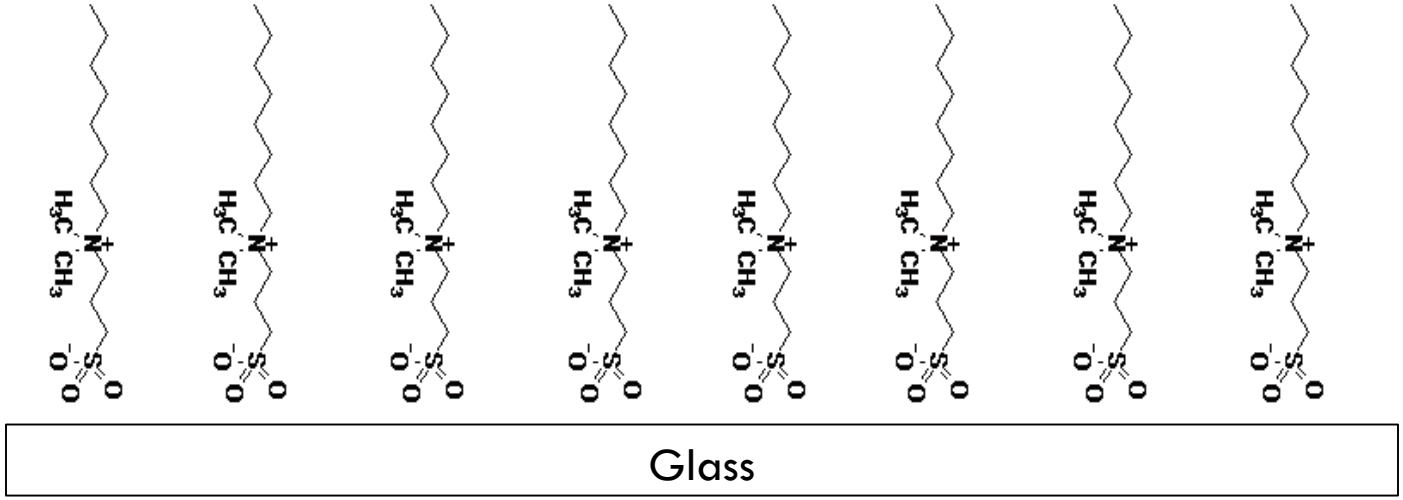


Figure 2. Electrophoretic separation of proteins with 100 mM PBS, pH 9, containing (a) 5 mM SB3–8, (b) no zwitterionic surfactants. See Fig. 1 for experimental conditions and labels.

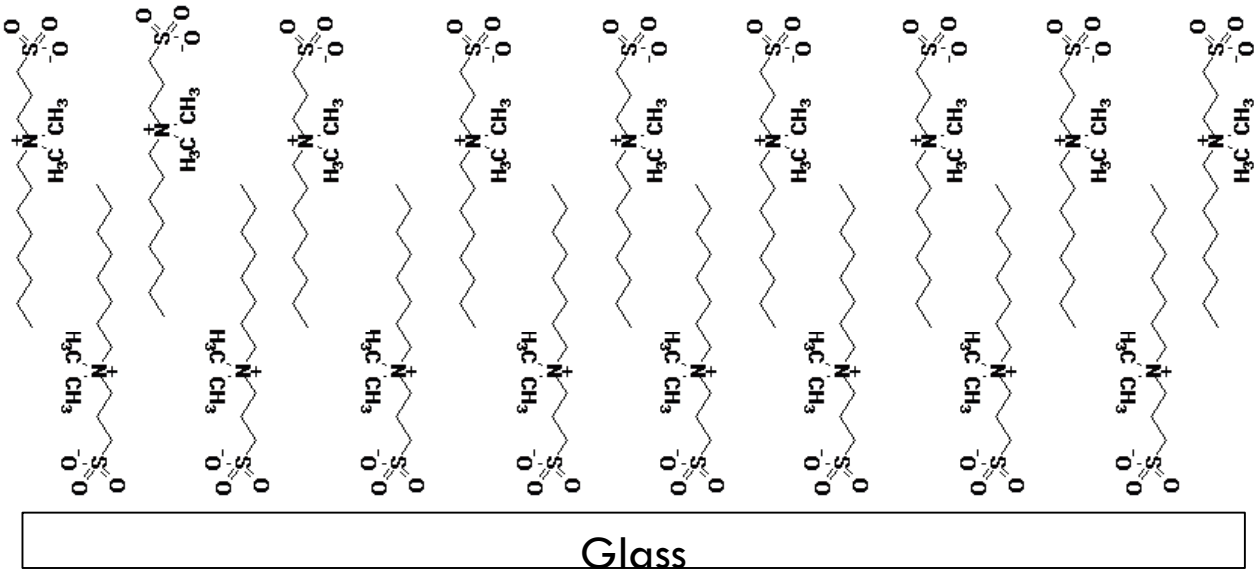
Gong and Ho Explanation

- Zwitterionic surfactant binds to charges on the glass channels
- Creates hydrophobic surface which repels water



Dr. Corcoran's Explanation

- Creates a type of bi-layer
  - Comparable to organic membranes
- Exposes Zwitterionic head groups which creates a solvated surface that acts like water

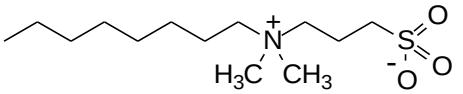


Dr. Shaoyi Jiang, University of Washington

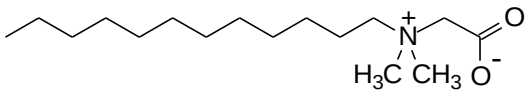
- Work done on developing non-fouling services
  - Used zwitterionic coatings with varying number of carbons between charges
  - Found that the greater the number of carbons, the less effective the coating

Surfactants

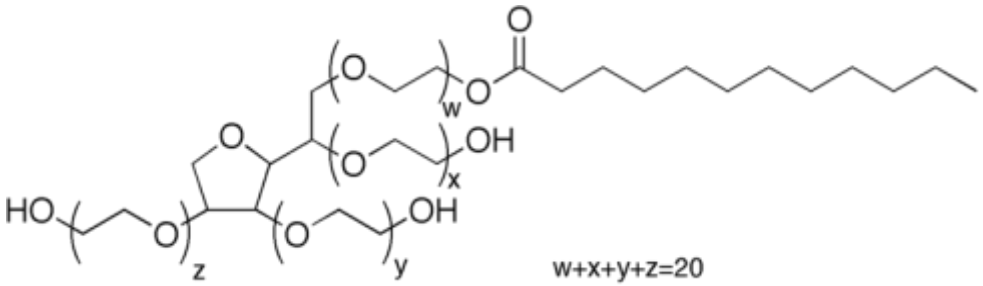
- Three Surfactants to be used
  - SB3-8, used by Gong & Ho
  - DMG-12, synthesized by Dr. Corcoran and myself
  - TWEEN 20, not zwitterionic like other compounds



SB3-8



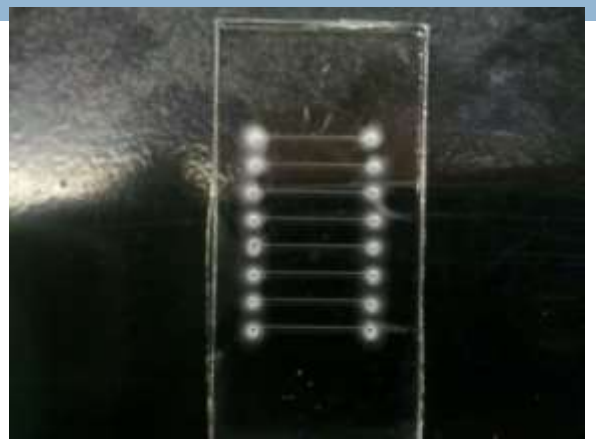
Corcoran's DMG-12



w+x+y+z=20

TWEEN 20

The Project



- Two Phases
  - 8 straight channel glass chip
    - Each channel 30µm deep
    - Tests done in static conditions
    - Measure fluorescence changes over time
  - Cross channel chip
    - Channel 10µm deep
    - Tests done with electroosmotic flow (EOF) present
    - Measure the velocity of dye over 1cm

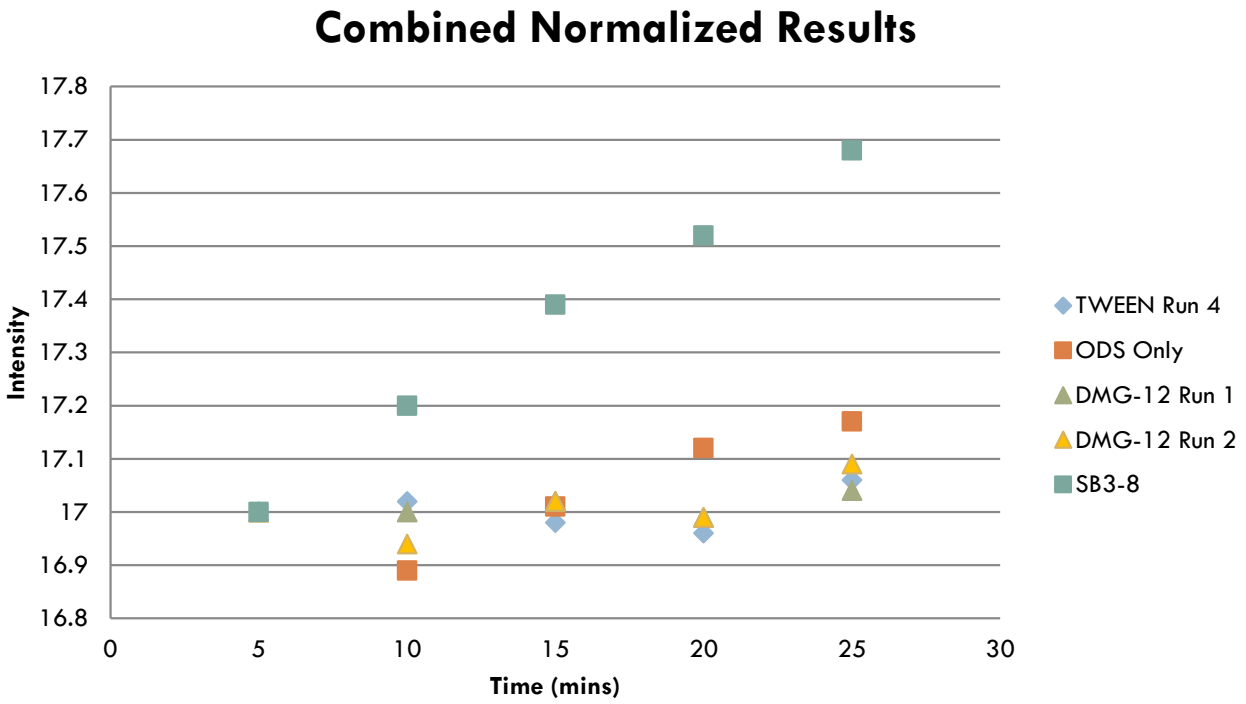
The Project, Phase 1

- Octadecylsilane (ODS) was used to mimic the bottom layer bound to the glass
  - ODS binds covalently rather than being ionically complexed
  - Completely eliminates the charge from the glass surface
  - Eliminates electroosmotic flow due to non-dynamic coating
- ODS coating washed with a surfactant
  - Gives the bi-layer type membrane proposed by Dr. Corcoran
  - Surfactant zwitterionic head becomes what is exposed

The Experiment

- TWEEN 20, SB3-8, and DMG-12 were applied to the ODS coating
  - Surfactants were applied with an HRPase-Streptavidin
  - Amplex Red/Hydrogen Peroxide added
    - Converts to a fluroscent dye, resorufin, when present with HRPase
- Fluorescence monitored under laser microscopy

Results

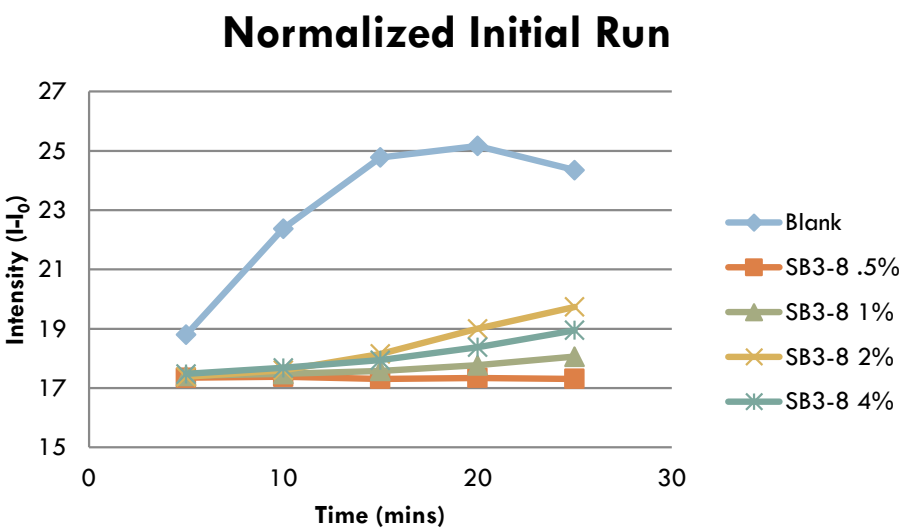


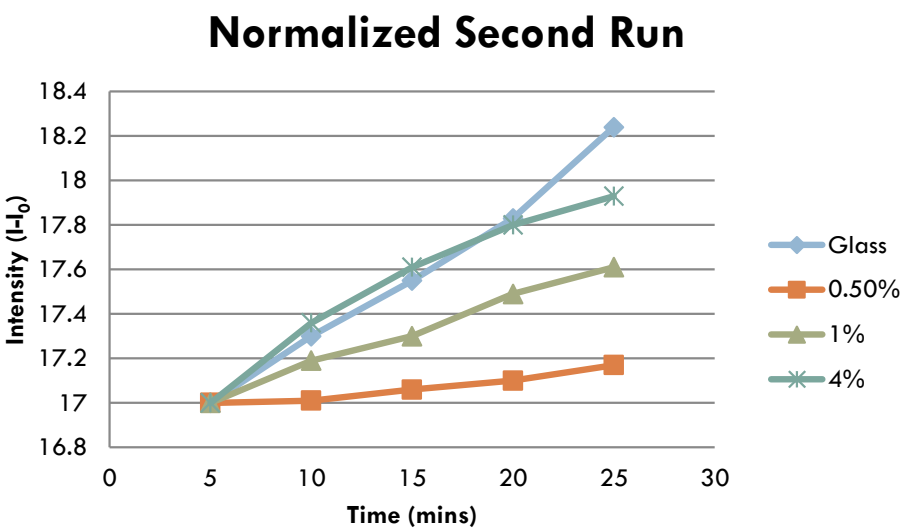
Time	ODS Only	TWEEN Run 4	DMG-12 Run 1	DMG-12 Run 2	SB3-8
5	17	17	17	17	17
10	16.89	17.02	17	16.94	17.2
15	17.01	16.98	17.02	17.02	17.39
20	17.12	16.96	16.99	16.99	17.52
25	17.17	17.06	17.04	17.09	17.68
Slope	0.019	0.0012	0.0014	0.0046	0.0336
R^2	0.970952125	0.060810811	0.30625	0.445286195	0.993522951

Phase 1, Continued

- Switched from covalent ODS coating to dynamic SB3-8 coating
  - Channel prepared in same way as with ODS coating
  - Bare glass, 0.5, 1, 2, 4% SB3-8 solutions were used

Results

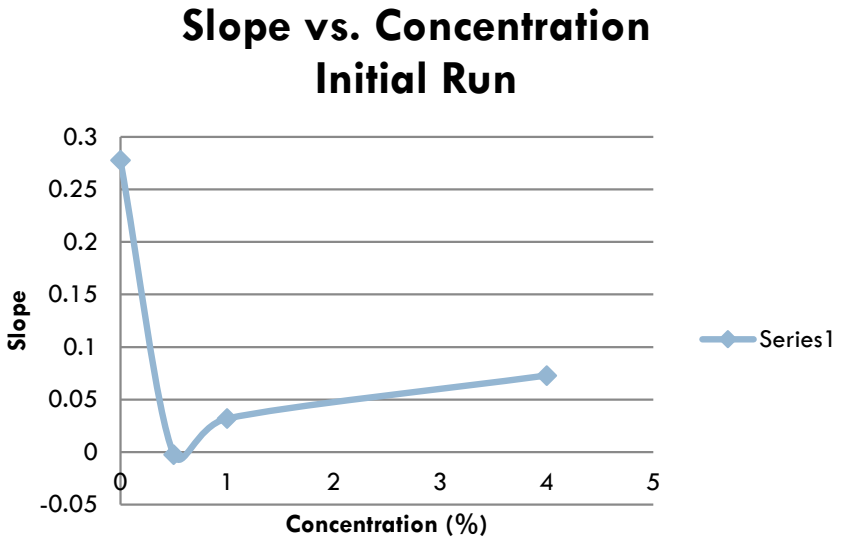




Time	Glass	SB3-8 .5%	SB3-8 1%	SB3-8 2%	SB3-8 4%
5	18.8	17.35	17.41	17.38	17.48
10	22.38	17.38	17.48	17.6	17.68
15	24.78	17.31	17.58	18.15	17.94
20	25.17	17.34	17.77	19	18.38
25	24.35	17.31	18.06	19.74	18.95
Slope	0.2778	-0.0024	0.0318	0.1224	0.0728
R^2	0.697056	0.413793	0.924689	0.961572	0.955739

Time	Glass	0.50%	1%	4%
Molarity	0	0.0178	0.0356	0.1424
5	17	17	17	17
10	17.3	17.01	17.19	17.36
15	17.55	17.06	17.3	17.61
20	17.83	17.1	17.49	17.8
25	18.24	17.17	17.61	17.93
Slope	0.0602	0.0086	0.0304	0.046
R^2	0.991562	0.949179	0.992952	0.964273

Interesting Trend Data



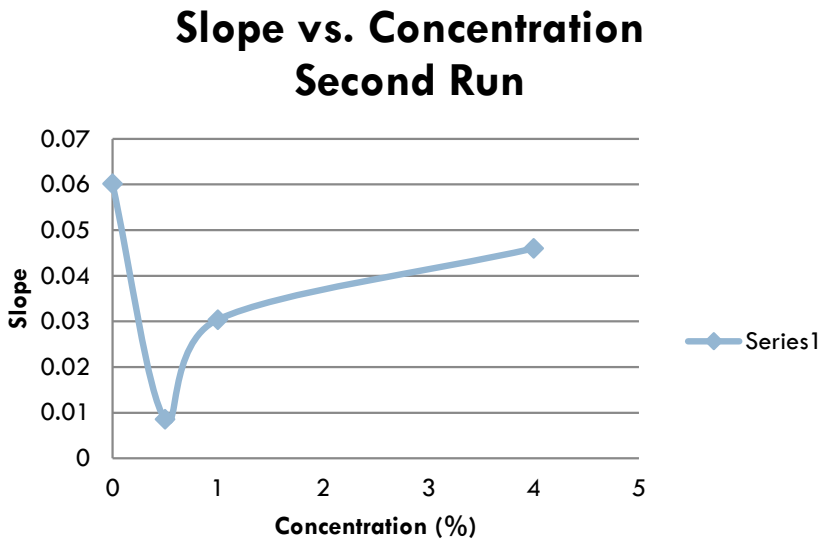


Table 1. Electrophoretic mobility of lysozyme in 40 mM phosphate buffer saline containing different amounts of SB3–8[a)]

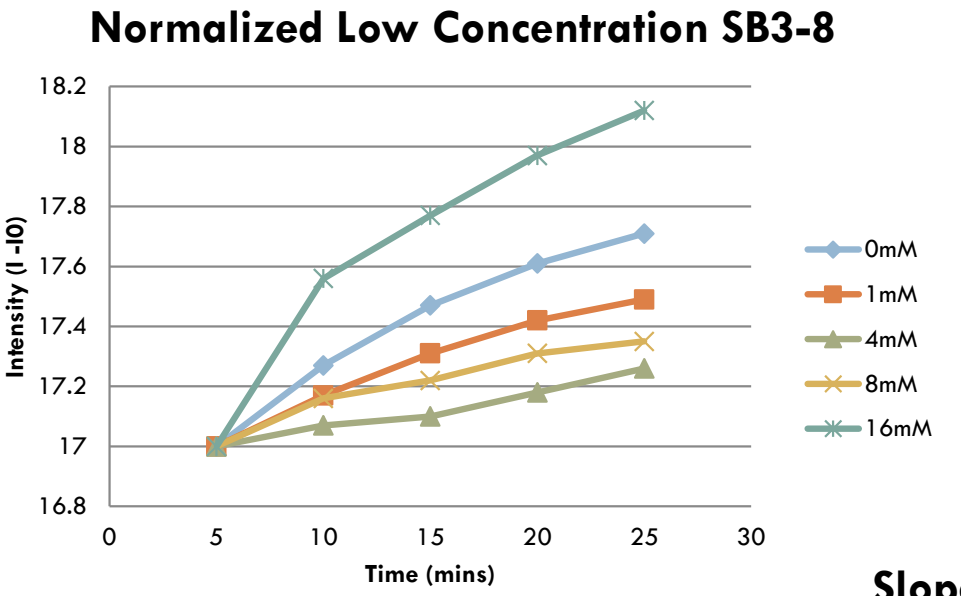
Buffers	Migration time min	Asymmetry	*N*(plate/m)
0 mM	7.94	32.1	917
5 mM	7.52	23.1	1079
10 mM	7.47	21.9	1163
20 mM	6.92	20.6	1321
40 mM	6.21	14.4	1800
60 mM	5.25	11.5	2783
80 mM	4.69	9.0	3629
100 mM	4.38	6.7	3288
200 mM	5.10	5.7	5496
400 mM	5.44	2.9	7096

a) Experimental conditions: 10 p.s.i./s injection pressure, 10 kV; SB3–8: *N*-octyl-*N*,*N*-dimethyl-3-ammonio-1-propanesulfonate

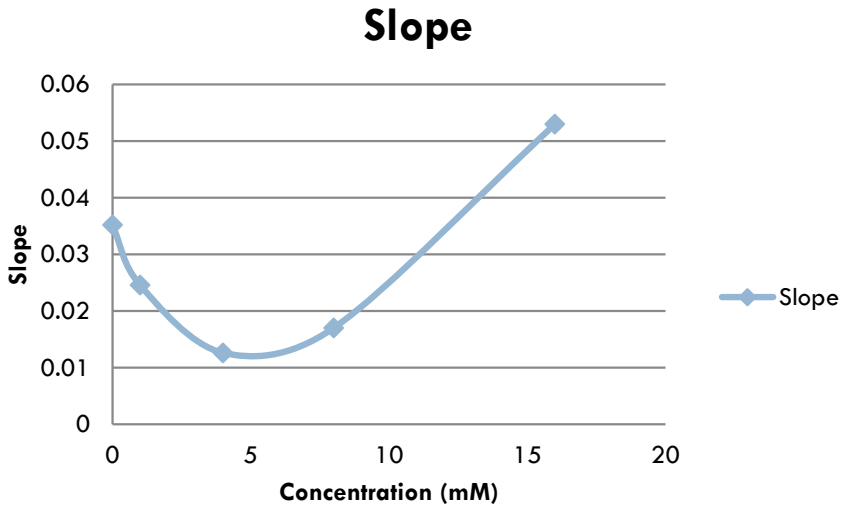
Phase 1, Continued

- 0.5% or 17.8mM appeared to be minimum seen in the current data
- Performed tests again using concentrations from 0-16mM

Results

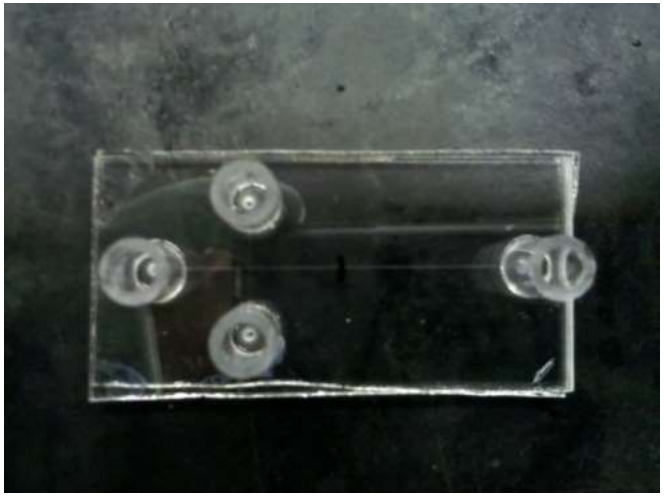


Time	0mM	1mM	4mM	8mM	16mM
5	17	17	17	17	17
10	17.27	17.17	17.07	17.16	17.56
15	17.47	17.31	17.1	17.22	17.77
20	17.61	17.42	17.18	17.31	17.97
25	17.71	17.49	17.26	17.35	18.12
Slope	0.0352	0.0246	0.0126	0.017	0.053
R^2	0.964143	0.975561	0.980484	0.947168	0.920959



Phase 2

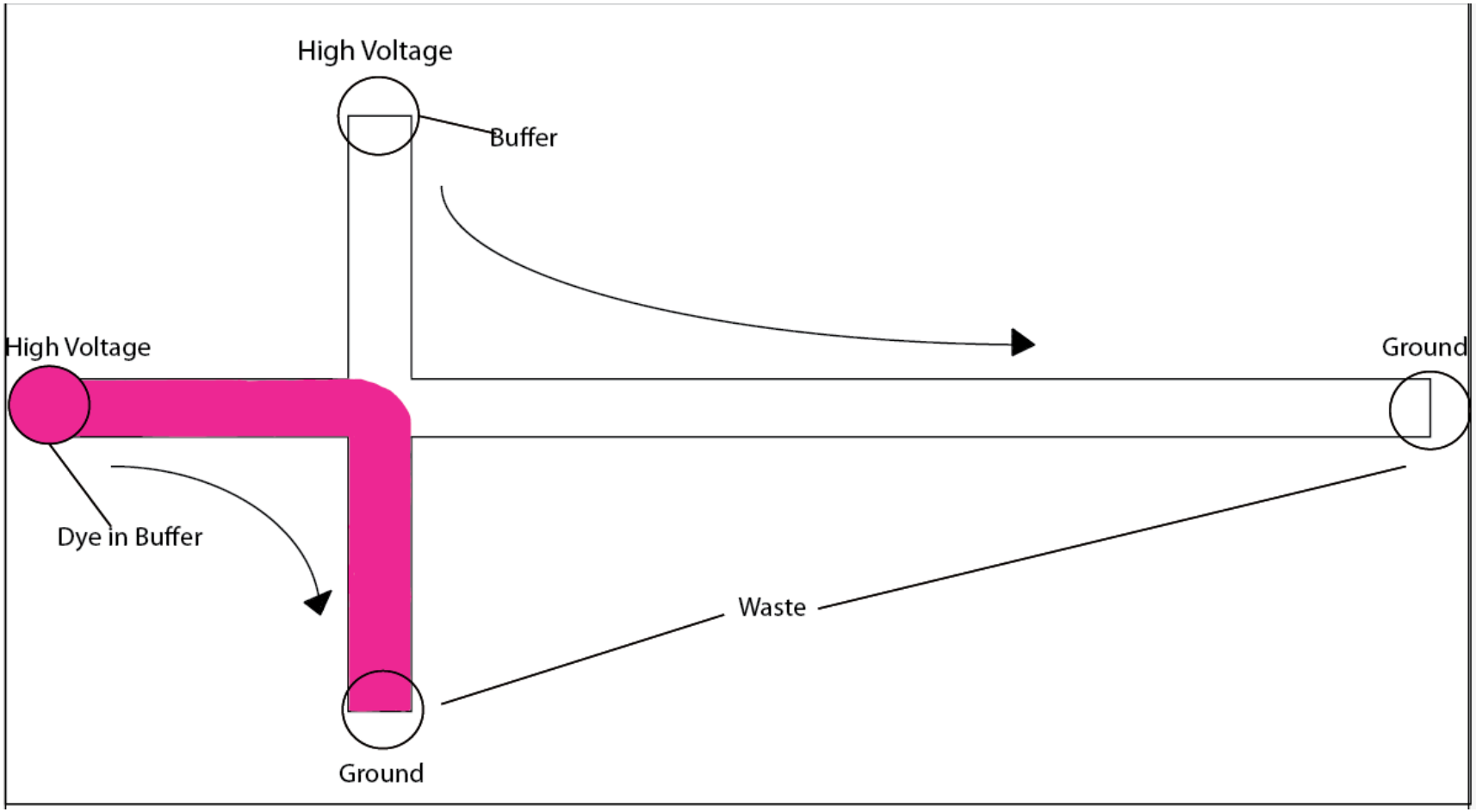
- Test the application of surfactant under conditions with flow, EOF
- Measured the time for neutral dye to travel 1 cm on bare glass, 1, 4, 8, 12, and 16mM coatings
- Was done using cross channel chip with a depth of 10µm.



How Surfactants Will be Tested

- 2 electrodes were used to generate the EOF
- One electrode was turned off briefly, 0.5 seconds
- Time it takes for dye plug to travel 1cm was measured for each surfactant

How Surfactants Will be Tested



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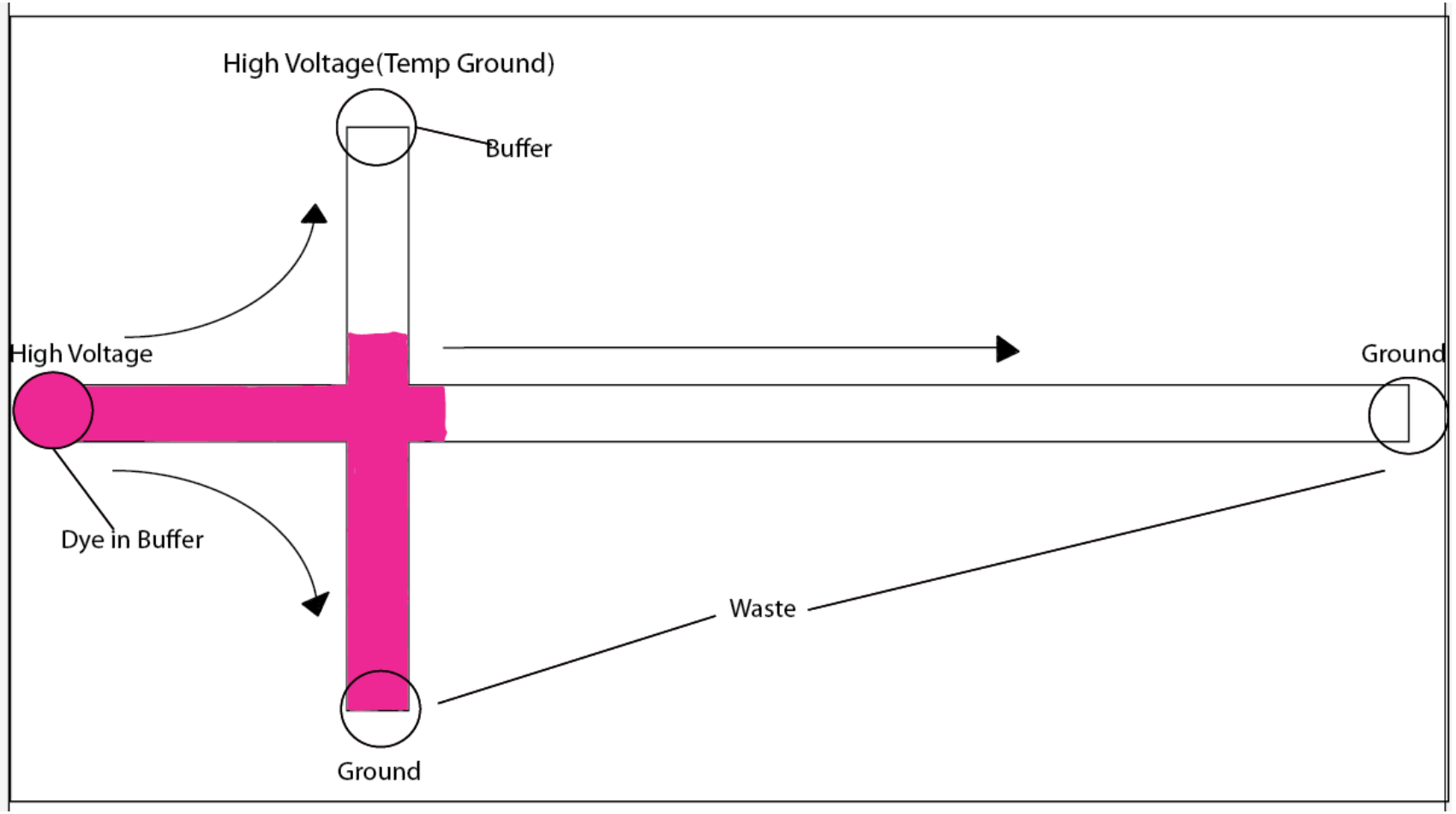

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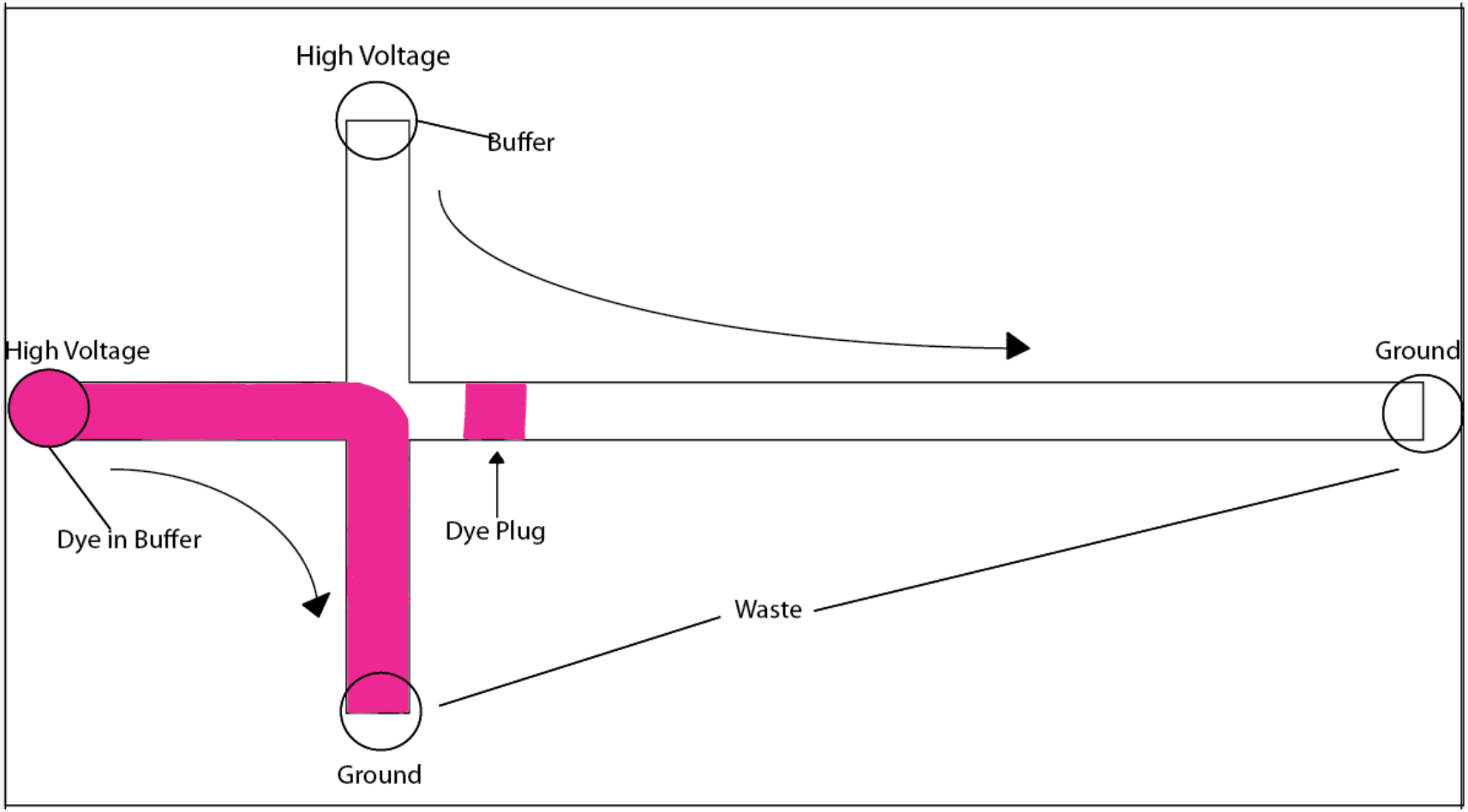
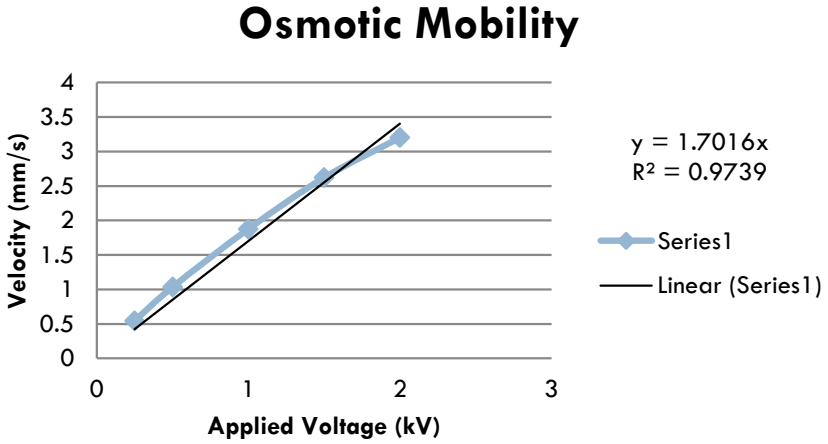


Phase 2 Testing

- Ran a 4mM coating at different voltages to get a sense of velocity

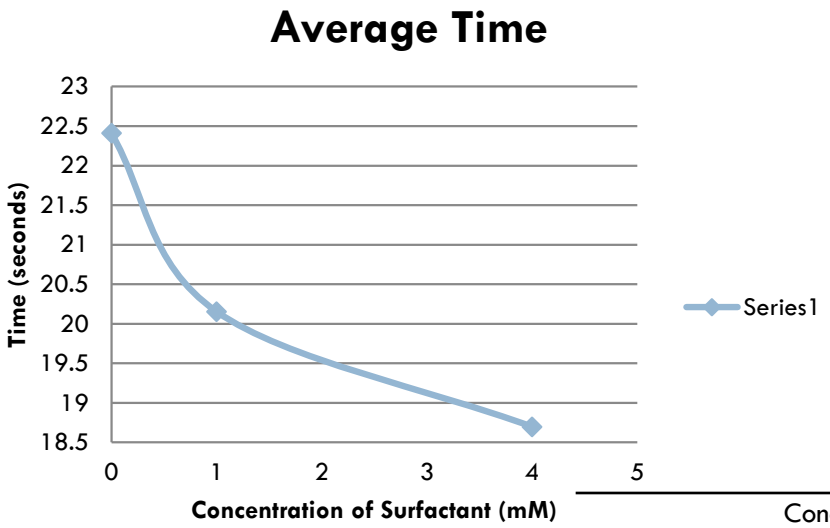


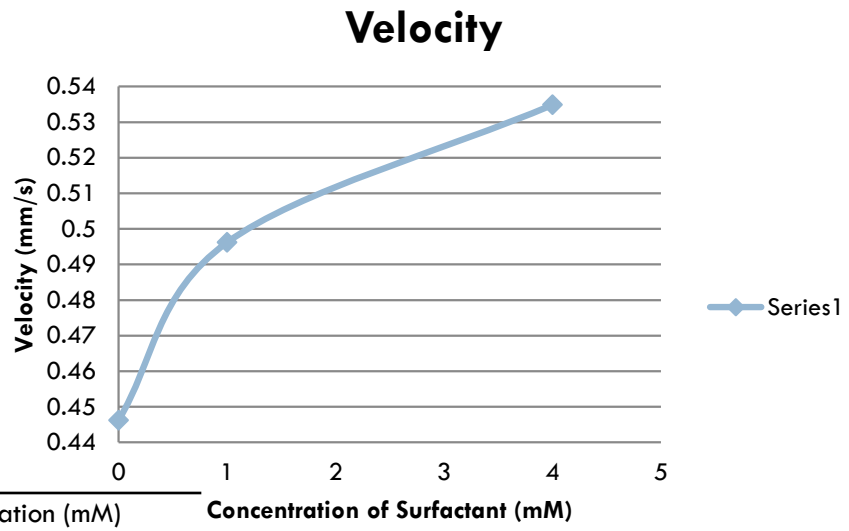
	Applied Voltage (kV)				
	0.25	0.5	1	1.5	2
Run	Time (Seconds)				
1	17.98	9.42	5.4	3.78	3.11
2	18.42	9.58	5.28	3.8	3.15
3	18.49	9.98	5.3	3.86	3.11
Average	18.29667	9.66	5.326667	3.813333	3.123333
v (mm/s)	0.546548	1.035197	1.877347	2.622378	3.201708

Phase 2 Testing

- Next step was to run full test starting with bare glass and increasing to 1, 4, 8, 12, and 16mM coatings
- Never made it past 1mM surfactant coating
  - After applying 4mM coating saw times around 25 seconds
  - Washed several times with 2mM PBS solution
  - Got near original values

Results





	Concentration (mM)		
	0	1	4
Run	Time (Seconds)		
1	22.36	20.08	18.4
2	22.33	20.16	18.74
3	22.54	20.22	18.89
			18.83
			18.57
			18.7
			18.77
			18.78
			18.58
Average	22.41	20.15333	18.69556
Velocity (mm/s)	0.446229	0.496196	0.534886

Problems That Still Remain

- Attempting to return to bare glass or 1mM surfactant with quick 2mM PBS solution did not appear to work
- Several different methods were attempted to remove coating
  - 2mM PBS with EOF or vacuum
  - NaOH, DI H_2O, THF, Acetonitrile and 2mM PBS with EOF, vacuum or static conditions
  - Multiple 15 minute static washes with 2mM PBS

Conclusions & Future Research

- Phase 1 results demonstrate the superior ability of DMG-12 compared to SB3-8
- Phase 2 EOF results give support to the work done by Gong & Ho
- Future Phase 2 testing done with DMG-12, is expected to demonstrate the same superior NSB prevention ability as in phase 1
- More work would need to be done on washing to make chips reusable for more consistent and comparable results

Acknowledgments

- Dr. Robert Corcoran and group
- Dr. Debashis Dutta and group
- UW Chemistry Department
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