Asymmetric Flow Field Flow Fractionation (AF4) of Aqueous C_{60} Aggregates with Dynamic Light Scattering Size and LC-MS

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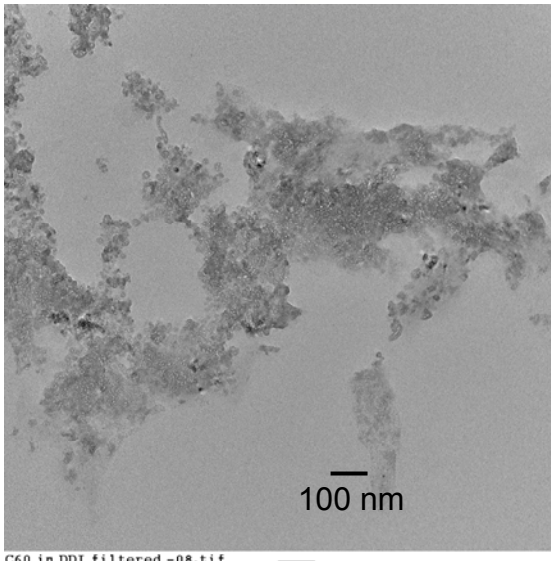
Fullerene Nanomaterials

- Used in medical,[1,2,3,4] cosmetic,[5,6,7] and electronic,[8,9] applications
- Very low water solubility for nonderivatized
- Aggregation in water (diameter <10- >500 nm)[10,11,12]
- Interest is growing in the transport and biological effects of fullerenes

C_{60}



(1)J. Am. Chem. Soc. 2005, 127, 12508-12509, (2)Antimicrob. Agents Chemother. 1999, 43, 2273, (3)VC60.com (4) J. Am. Chem. Soc.; 1993; 115, 7918-7919, (5) Burangulov, N. *et al,* Cosmetic compositions containing fullerene clusters, Patent WO2006/001784, (6) sircuitskin.com (7) zelens.com (8) Nature 1991, 350, 320-322 (9) J. Chem. Inf. Sci. 1999, 39, 180-191 (10) *Environ. Sci. Technol.* **2005,** 39, 6343-6351 (11) *Langmuir* **2001,** 17, 6013-6017. (12) *Environ. Sci. Technol.* **2005,** 39, 4307-4316.

What is needed?

- Methods are needed which can size separate aggregates in the nanometer size range[1,2]
- Methods need to be able to distinguish between natural and fullerene materials[1,2]

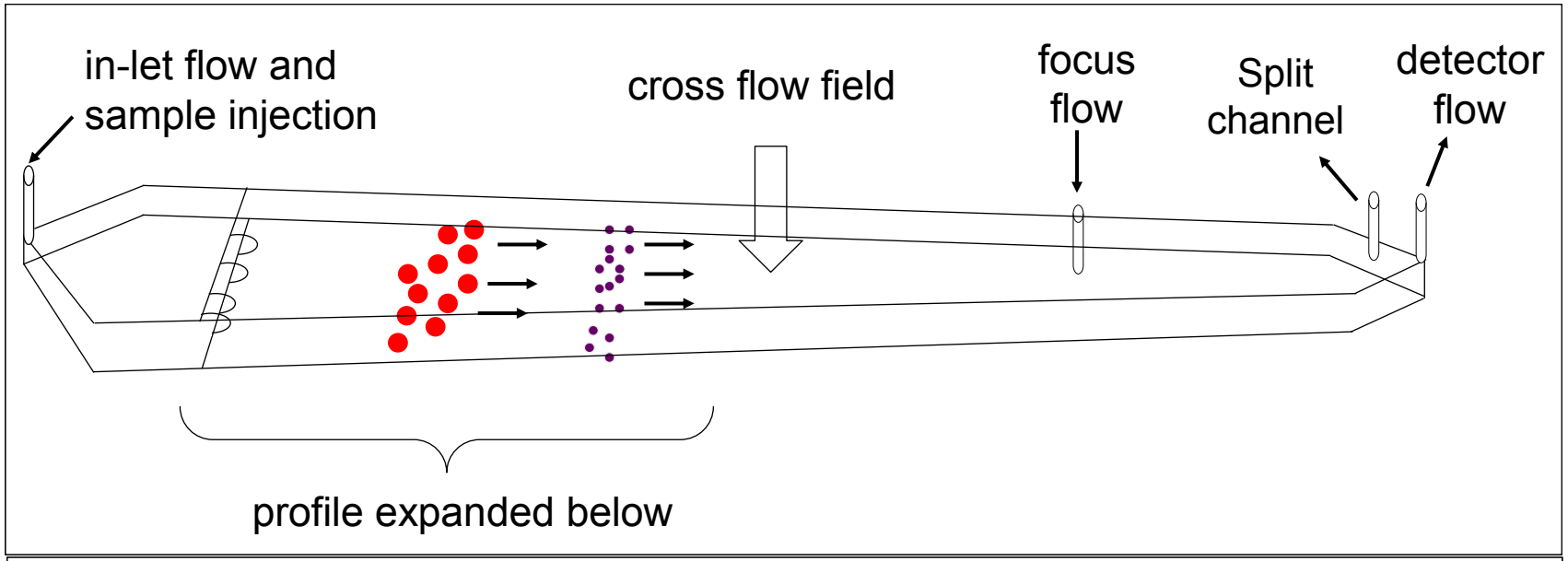
1) EPA Nanotechnology White Paper 2) NNI EHS research needs for engineered nanoscale technologies

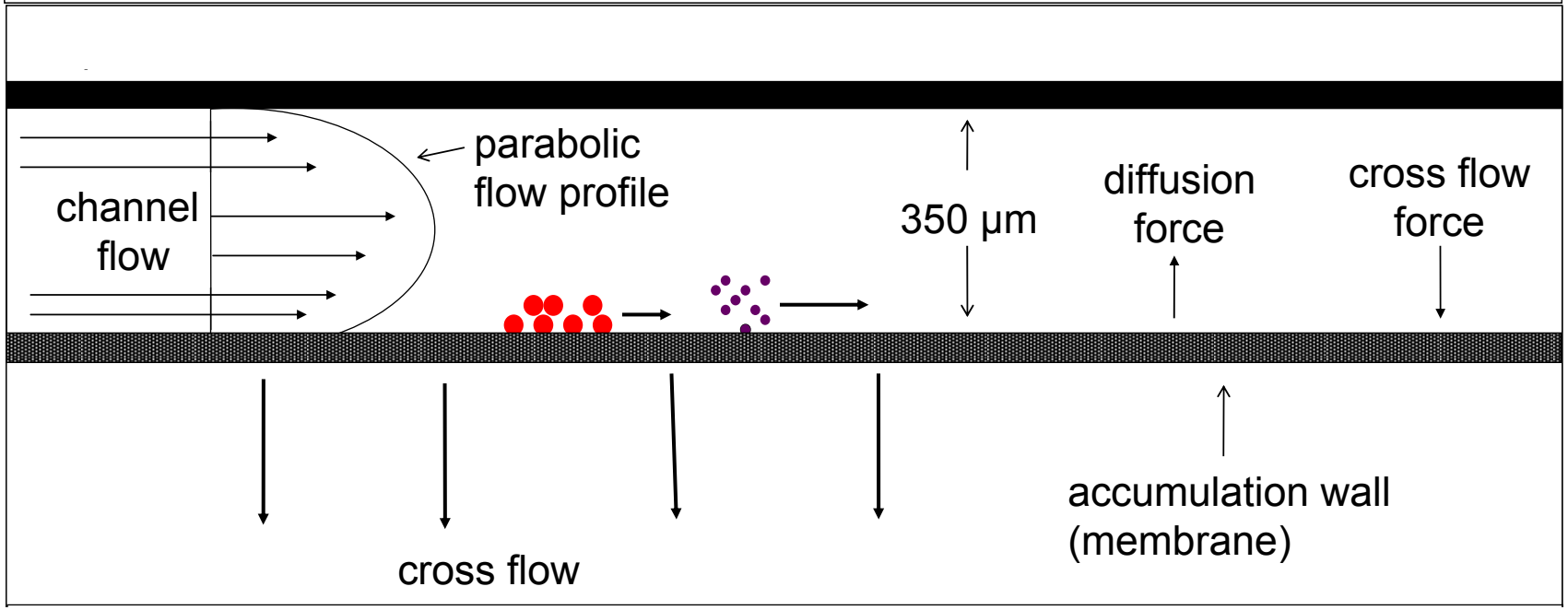
Why Asymmetric Flow Field-Flow Fractionation (AF4)

- Able to separate materials in the nanometer size range, 1-500 nm diameter[1,2,3]
- Versatile range of aqueous mobile phases:
  - Up to 10 mM ionic strength[1,2,3]
  - With and without surfactants[1,2,3]
  - Eluent and sample solution chemistry the same[1,2,3]
- Fractions can be collected for further analysis

1) Field-Flow Fractionation Handbook, 2000 2) Anal. Chem. 59, 1332-1339 3) Science, 260, 1456-1465.

Briefly, AF4


in-let flow and
sample injection
cross flow field
focus
flow
Split
channel
detector
flow
profile expanded below


channel
flow
parabolic
flow profile
350 µm
diffusion
force
cross flow
force
cross flow
accumulation wall
(membrane)

Approach to aqu/C_{60} Quantification

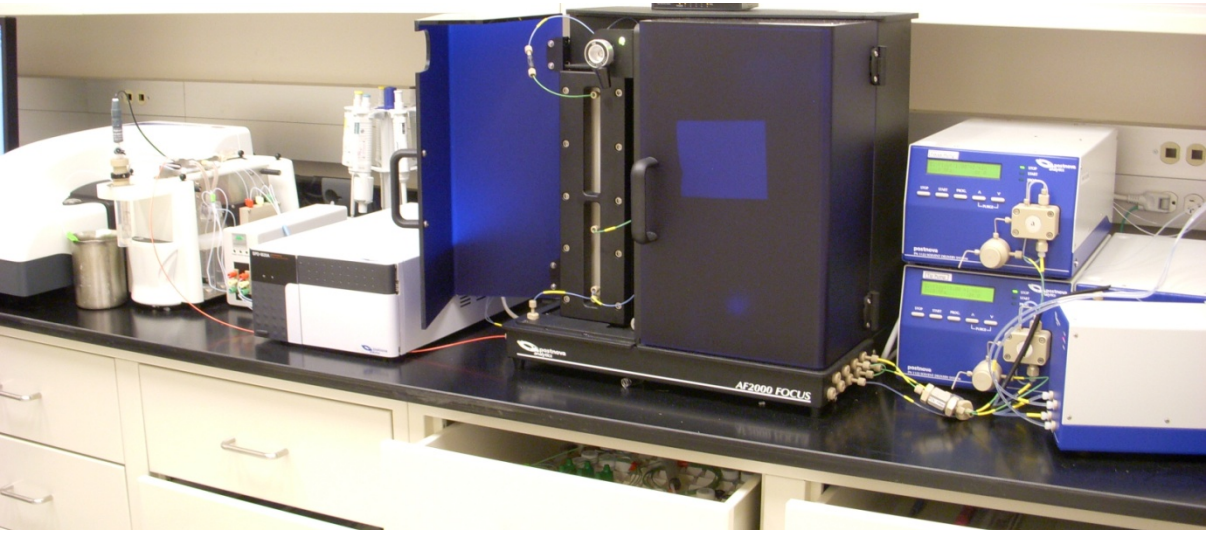
- Fractions collected every 2 minutes from AF4
- C_{60} extracted into toluene by salting out with NaCl
- Quantified by liquid chromatography atmospheric pressure photo-ionization mass spectrometry (LC-APPI-MS) with $^{13}C_{60}$ internal standard[1,2]

- Accela liquid chromatograph (LC), (Thermo Fisher) with a cosmosil (150 mm x 4 mm) column with a 95% toluene 5% methanol mobile phase.
- Quantum Ultra triple quadrapole mass spectrometer (MS), (Thermo Fisher)
  - negative atmospheric pressure photo-ionization
  - Quantify molecular ions C60 (m/z 720) and $^{13}C_{60}$ (m/z 734)

1) Anal. Chem. (23) 9091-9097
2) 2) Rapid Commun. Mass. Spectrom. (20) 2783-2785

Instrumentation

- AF4 Postnova (Salt Lake City, UT)
- Malvern Zetasizer NanoZS Dynamic Light Scattering (DLS)



•Nonlinear elution programs were used

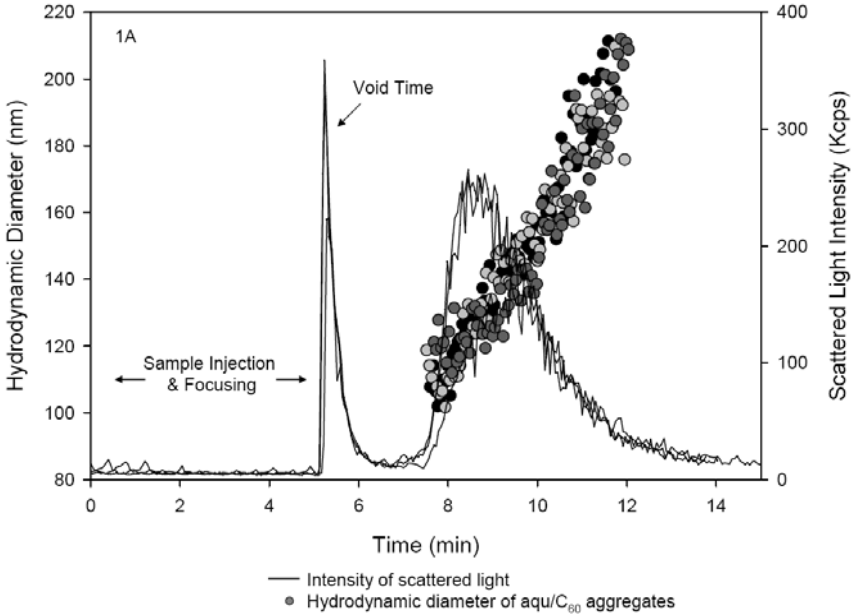
- AF4 modified with:
  - 50, 100, 500, 1,000 and 2000 µL injection loops
  - 350 µm spacer
  - 10 kDa polyethersulfone and cellulose acetate membranes (Postnova)
  - Polypropylene membrane, 30 nm pore size (Celgard 2320)
  - 10 nm pore size polycarbonate membrane (GE)

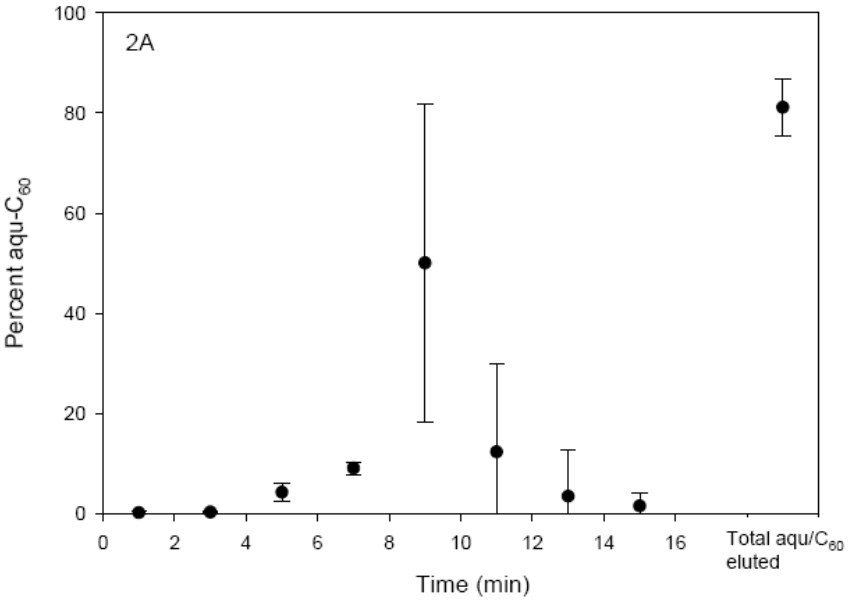
Suspension Generation

- Aqu/C_{60} generated by sonication at 300 watts for two 5 minute periods in deionized H_2O (GENEQ, Montreal, QC)
- Filtered through 0.45 μm cellulose acetate filter (Chrom Tech)
- No solvents used
- Initial suspension:
  - Hydrodynamic diameter (D_h) of 130 ± 1 nm
  - Zeta potential -40 ± 1 mV
  - Mass suspended 1.7 ± 0.12 mg C_{60}/L

Fractionation at 1 mL/min Cross Flow

- 4.2 ± 1.8 % of aqu/C_{60} elute at void time
- Predicted retention times of 11.5 and 13.4 min retention times for 110 and 200 nm D_h [1]
- 9.1 ± 1.3 % of aqu/C_{60} less than 110 nm in D_h
- 76 ± 7.5 % of aqu/C_{60} eluted
  - PES PZI pH=3.1[2]
  - Van der Waals and steric interactions may influence deposition

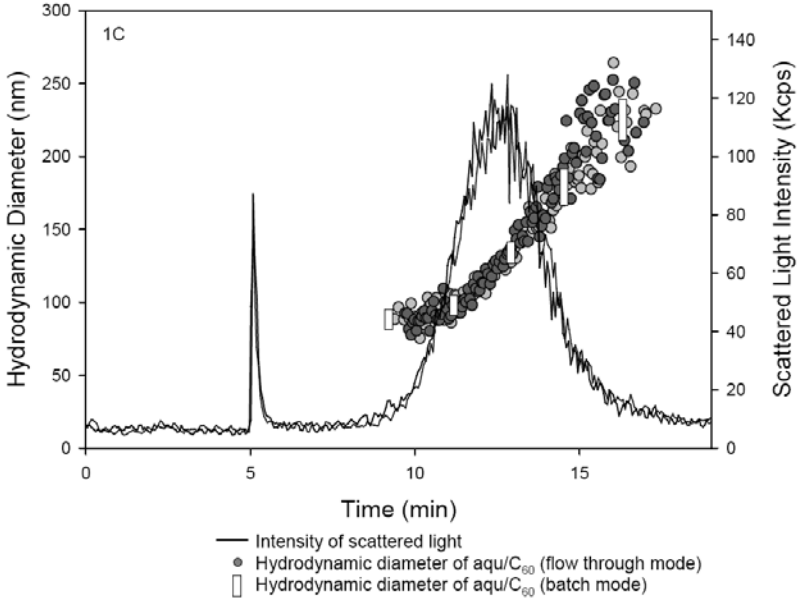


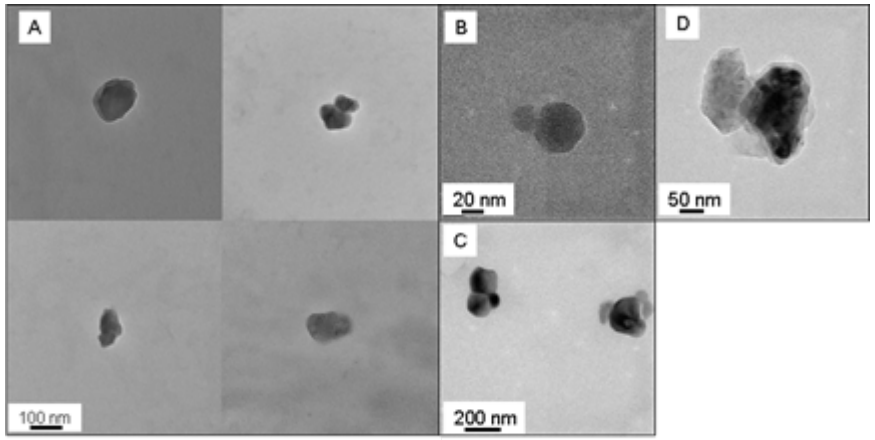


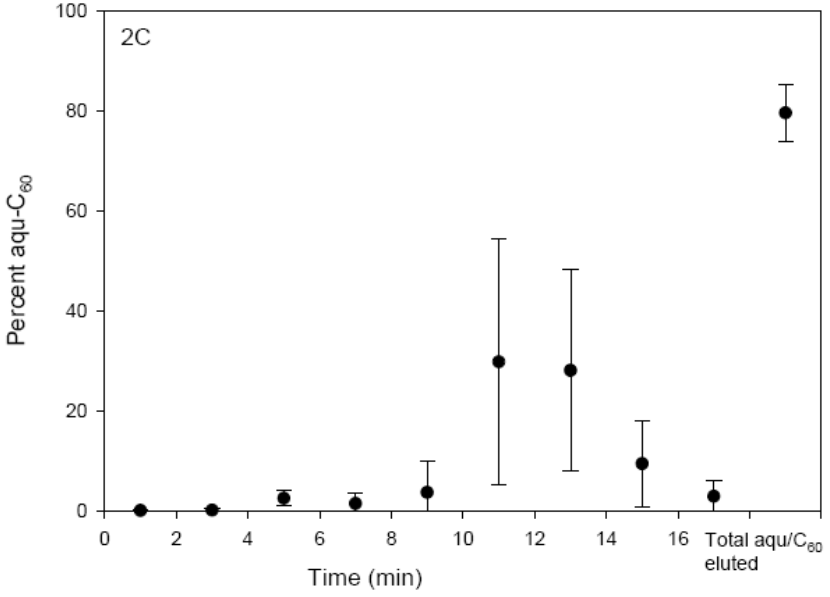
1) J. Chrom. A, 1134, 236-245.
2) J. Membrane Sci 129, 125

Fractionation at 4 mL/min Cross Flow

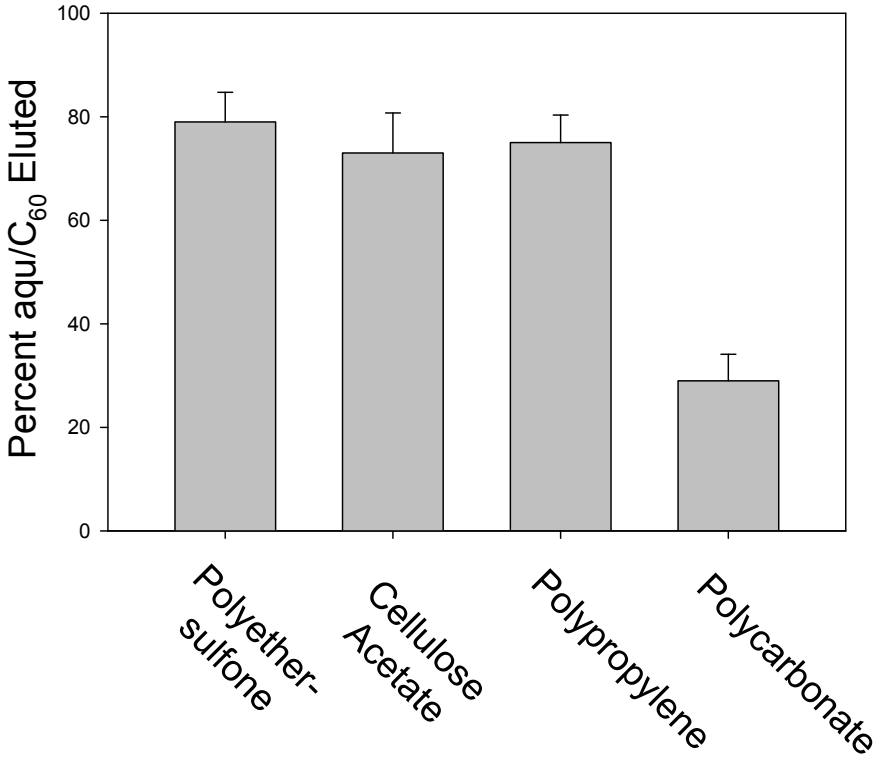
- Aggregate sizes 80-260 nm
- 2.5 ± 1.5 % of aqu/C_{60} elute at void time
- 5.2 ± 6.7 % of aqu/C_{60} < 80 nm
- 77 ± 5.8 % of aqu/C_{60} eluted
- Sizes corroborated by DLS in batch mode and TEM



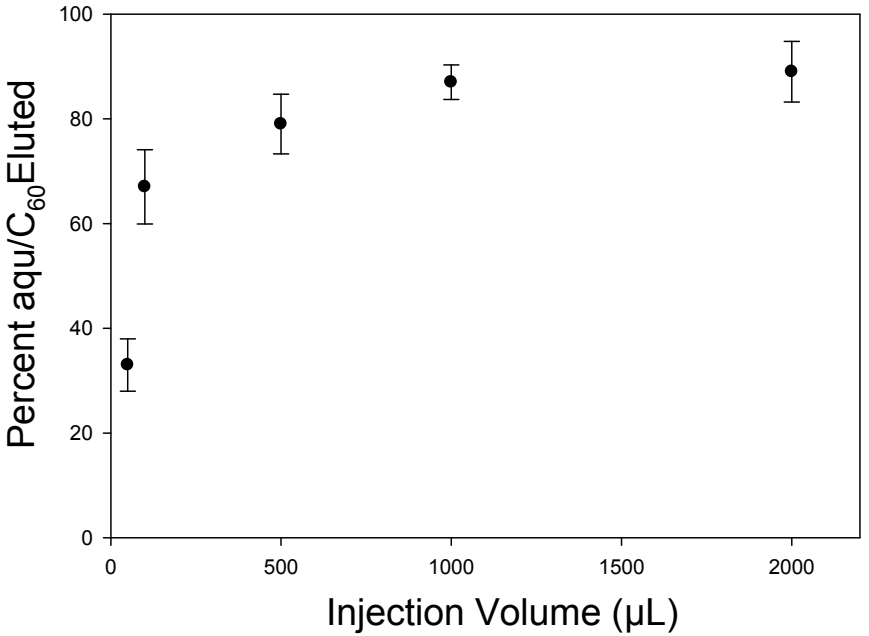




Effect of Injection Volume and Membrane Type



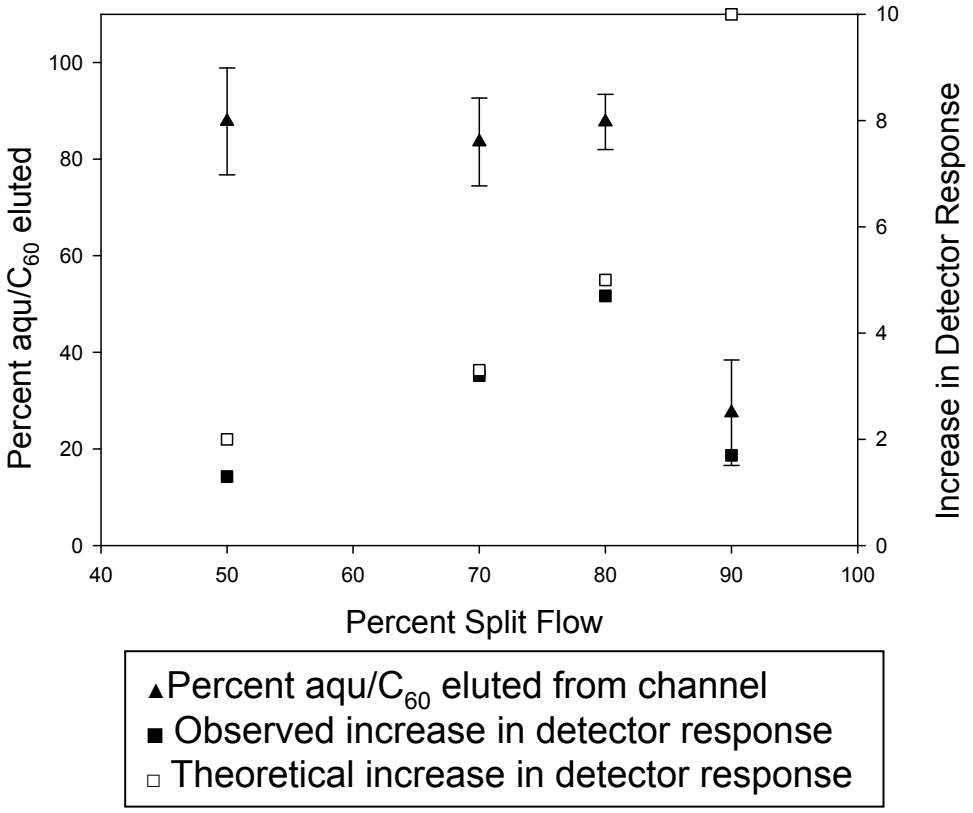
- Alternative membranes do not decrease the amount of deposition observed
- Increased deposition on polycarbonate membrane likely due to surface heterogeneities



- Low recoveries at smaller injection volumes and higher recoveries at larger injection volumes
- Deposition due to limited number of sorption sites on each membrane

Increasing Detector Response with Split Channel Flow

- At up to 80% of channel flow is split to waste:
  - ~ 80% of the aqu/C_{60} elutes through detector
  - Detector response increases according to theory
- At 90% split to waste
  - 28 ± 11 % of aqu/C_{60} elutes through detector
  - Detector response only increases by 1.7x when 10x is expected
- Decreased response and recovery result from non-ideal flow in channel



Conclusions

- Aqu/C_{60} aggregates ranged in size from 80-260 nm in D_h as determined by AF4-DLS
- LC-MS was successful in quantifying the mass of aqu/C_{60} in each size fraction
- Deposition was observed, as only ~80% of aqu/C_{60} eluted from the AF4 channel
- Alternative membranes did not reduce deposition
- Decreasing the injection volume led to decreased recoveries
- Increased injection volume led to increased recoveries
- >80% split channel flow resulted in reduced detector response and reduce C_{60} recoveries

Acknowledgements

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