Size Determination of Aqueous C_{60} Colloids by Asymmetric Flow-Field Flow Fractionation (AF4) and in-Line Dynamic Light Scattering

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I. Abstract

A size separation method was developed for aqueous C_{60} fullerene aggregates (aqu/C_{60}) using asymmetric flow field-flow fractionation (AF4) coupled to a dynamic light scattering (DLS) detector in flow through mode. Surfactants, which are commonly used in AF4, were avoided as they may alter suspension characteristics. Aqu/C_{60} aggregates generated by sonication in deionized water ranged in hydrodynamic diameter (D_h) from 80-260 nm as determined by DLS in flow through mode. Size observations were confirmed by DLS (batch mode) and TEM techniques. The mass of C_{60} in each fraction was determined by LC-APPI-MS. Of the total aqu/C_{60} mass, only 7.7 ± 6.9 % had D_h less than 80 nm, while 58 ± 32 % had D_h between 80-150 nm and 14 ± 9.2 % were between 150-260 nm in D_h. With the optimal fractionation parameters, 79 ± 5.7 % of the aqu/C_{60} mass eluted from the AF4 channel, indicating deposition occurred during fractionation. Use of alternative membranes did not reduce deposition. Channel flow splitting increased detector response, although not to the degree predicted by theory. This is the first report of the high resolution size determination of aggregated aqu/C_{60}.

II. Background

- Fullerenes are a widely used nanomaterial [1,2,3,4,5]
- Concern is growing over the impact a potential release of fullerenes may have on human and environmental health[6,7]
- Aqueous suspension of nanometer sized fullerene aggregates can be formed by sonication, extended stirring and solvent exchange [8,9,10]
- Current light scattering techniques are limited by poor resolution and sample heterogeneity
- Microscopy techniques are not suitable for large sample sizes and are prone to artifacts[10]
- AF4 is used to size separate polydisperse particle populations[11]
- In normal mode, AF4 can separate particles from 1 to 500 nm in diameter[11]
- Elution conditions can vary with ionic strength and surfactant type[11]
- AF4 coupled in-line with light scattering detectors allows for simultaneous size fractionation, detection and size determination of polydisperse colloids[12]

III. AF4 Size Separation

- In AF4 two flow streams effect separation:
  - the cross flow forces particles against the accumulation membrane
  - the channel flow sweeps particles to detector
- Small particles with large diffusion coefficients, diffuse further into the channel than large particles with small diffusion coefficients
- In the parabolic channel flow, small particles experience a greater channel velocity than large particles
- Small particles elute before large particles

IV. Objectives

- Develop an AF4 size separation method for aqu/C_{60} based on DI H_2O eluent
- Determine the C_{60} mass distribution within the aqu/C_{60} size distribution
- Determine effect of different membrane type and injection volume on fractionation efficacy

V. Experimental

- AF4 (AF 2000, Postnova, Salt Lake City, UT) coupled in-line to a DLS (Malvern, Westborough, MA)
- Used 50, 100, 500, 1000, 2000 µL injection volumes
- Membranes included cellulose acetate (10 kDa), polyethersulfone (10 kDa), polypropylene (30 nm) and polycarbonate (10 nm)
- Fractions were collected every two minutes and the mass of C_{60} in each fraction was determined by liquid chromatography-atmospheric pressure photoionization-mass spectrometry (LC-APPI-MS)
- Inline (DLS) size determinations were corroborated by analysis of fractions by batch mode DLS and TEM

VI. Results and Discussion

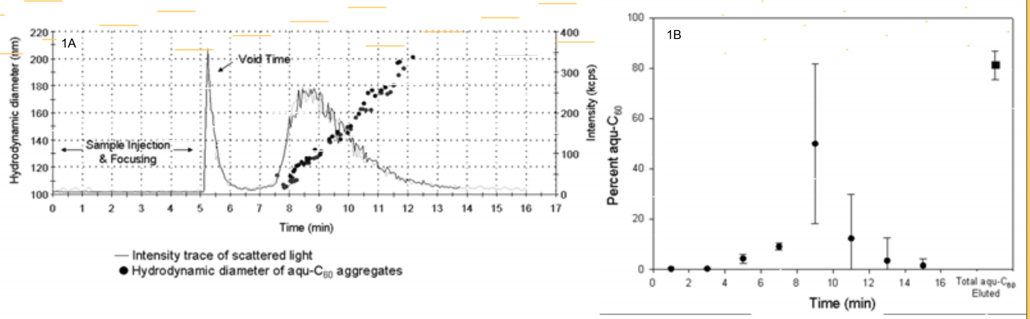


Figure 1A, AF4 fraction of aqu/C_{60} at a cross flow of 1 mL/min, channel flow 1 mL/min, with size determination by DLS indicates aqu/C_{60} elute in order of increasing size with D_h ranging from 110-200 nm. Figure 1B, fractions collected at two minute intervals (●) indicate that 13 ± 2.2 % have D_h less than 110 nm, 50 ± 32% have D_h between 110-150 nm and 17 ± 20 % have D_h between 150-200 nm. Deposition was observed as 81 ± 5.8 % (■) of the aqu/C_{60} eluted from the AF4 channel.

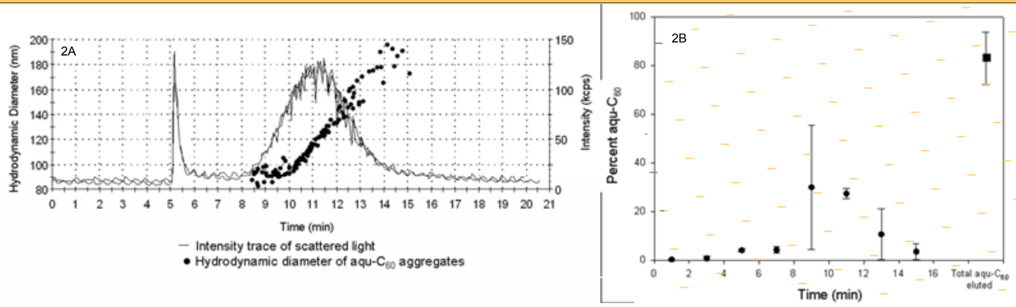
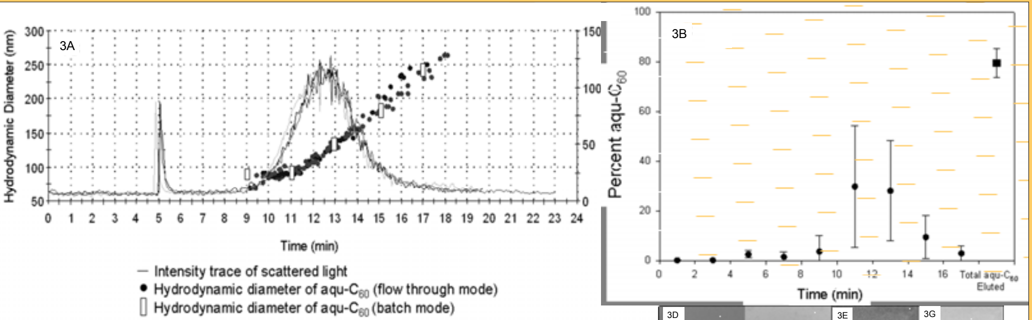


Figure 2A, Increased cross flow of 2 mL/min and channel flow 1 mL/min provides greater resolution and indicates aqu/C_{60} aggregates ranged from 80-200 nm in D_h. Figure 2B, fractions collected at two minute intervals (●) indicate that 8.3 ± 1.5 % of the aqu/C_{60} have D_h less than 80 nm, 57 ± 26 % have D_h between 80-130 nm and 14 ± 11 % have D_h between 130-200 nm. Deposition did not increase as 83 ± 11 % (■) of the aqu/C_{60} eluted from the AF4 channel.



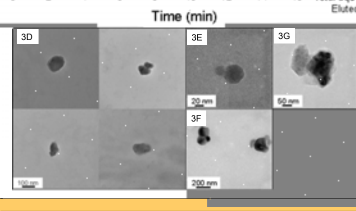


Figure 3A, Fractionations at a cross flow of 4 mL/min and channel flow 1 mL/min provide greater resolution, indicate aqu/C_{60} aggregates range from 80-260 nm in D_h. Figure 3 B, fractions collected at two minute intervals (●) indicate that 7.7 ± 6.9 % of the aqu/C_{60} have D_h less than 80 nm, 58 ± 32 % have D_h between 80-140 nm and 14 ± 9.2 % have D_h between 130-260 nm. Increased deposition was observed as 79 ± 5.7 % (■) of the aqu/C_{60} eluted from the AF4 channel. Size determinations were verified by batch analysis by DLS (3A) and TEM (3 D-G).

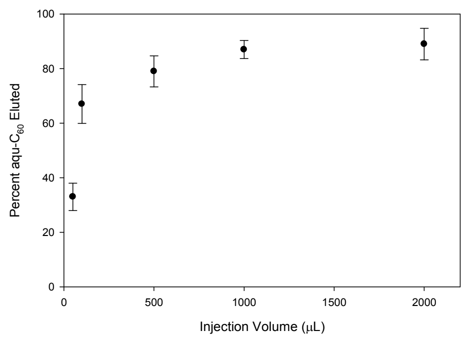


Figure 4, percent of aqu/C_{60} that eluted from the AF4 channel upon injection of varying amounts aqu/C_{60}. Achieving high recoveries at large injection volumes and low recoveries at low injection volumes indicates a limited number of sorption sites for aqu/C_{60}

Table 1, Recovery with Different AF4 Membranes

Membrane Type (Pore Size)	Recovery
Polyethersulfone (10 kDa)	79 ± 5.7 %
Cellulose Acetate(10 kDa)	73 ± 7.7 %
Polypropylene (30 nm)	75 ± 5.3 %
Polycarbonate (10 nm)	29 ± 5.1 %

Deposition in the AF4 Channel is not determined by membrane aromaticity, electron donating capacity, hydrophobicity, or pore size. The low recovery for the polycarbonate membrane likely results from surface heterogeneities.

VII. Conclusions

With the 4 mL/min cross flow fractionation program, aqu-C_{60}:

- Ranged in D_h from 80-260 nm
- Only 7.7 ± 6.9 % of the total aqu/C_{60} mass had D_h less than 80 nm
- 58 ± 32 % of the total aqu/C_{60} mass had D_h between 80-150 nm
- 14 ± 9.2 % of the total aqu/C_{60} were between 150-260 nm in D_h
- Deposition was observed as 79 ± 5.7 % of the aqu/C_{60} eluted from the AF4 channel

VIII. Acknowledgements

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