

Characterizing Dendrimers by Asymmetric Flow Field Flow Fractionation



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Why look at Dendrimers?

- Dendrimers have a high degree of molecular uniformity, narrow molar weight distribution, and specific size and shape (theoretically).
- Highly functionalized surface (-NH₂, -OH,...) for covalent conjugation.
- Dendrimers are used as controlled drug delivery vehicles.
- Conjugation with DNA for in-vitro gene transfection by electrostatic interactions.
- Low cytotoxicity due to their size.

Why light scattering?

- Measure absolute molar mass and size (rms radius, hydrodynamic radius) in solution.
- Can be easily combined with Size Exclusion Chromatography (SEC) or Field Flow Fractionation (FFF) separation methods.
- Determine fragments, aggregates, and stoichiometry of complexes.

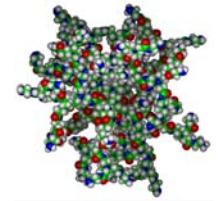
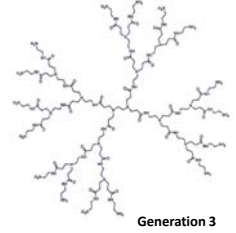
Why use FFF as a separation method?

- FFF does not have a stationary phase, gentle separation method.
- Separation of small molecules and large aggregates in one experiment.
- SEC requires high salt concentration and low pH when characterizing PAMAM dendrimers to prevent interaction with stationary phase. Typical mobile phases are 0.5 M NaNO₃ + 0.6 M HOAc at pH 2.7.
- Use PBS as elution buffer in FFF.

Ethylenediamine-core poly(amidoamine) (PAMAM) Dendrimers

Commercial samples obtained from Dendritech, Inc.

Generation	Molecular Weight	Measured Diameter (Å)	Surface Groups
0	517	15	4
1	1,430	22	8
2	3,256	29	16
3	6,909	36	32
4	14,215	45	64
5	28,826	54	128
6	58,048	67	256
7	116,493	81	512
8	233,383	97	
9	467,162		2048
10		135	4096

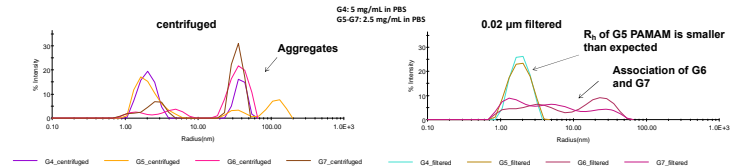


Theoretical Properties, not to be used as specifications

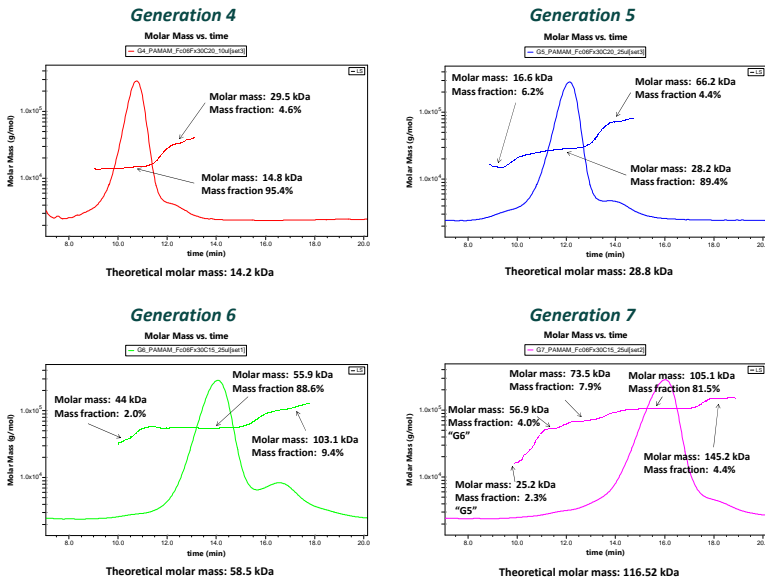
AF4 Experimental Conditions

- 5 kDa regenerated cellulose membrane, long channel
- Eclipse Dualtec with Heleos II + TrEX, QELS on detector 16
- Eluent: PBS (50 mM phosphate, 50 mM NaCl)
- Channel flow 0.6 mL/min, constant cross flow of 3.0 mL/min for 20 min
- Sample concentration: 5 mg/mL for G4 PAMAM, 2.5 mg/mL for G5-G7 PAMAM

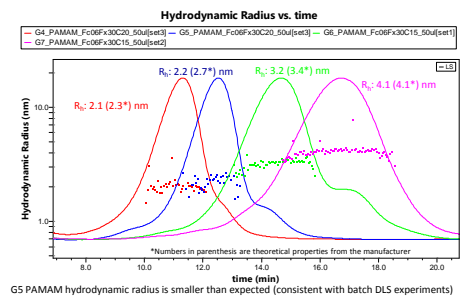
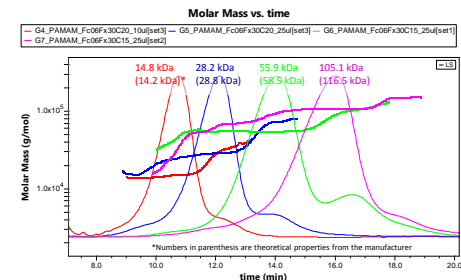
Batch DLS Measurements



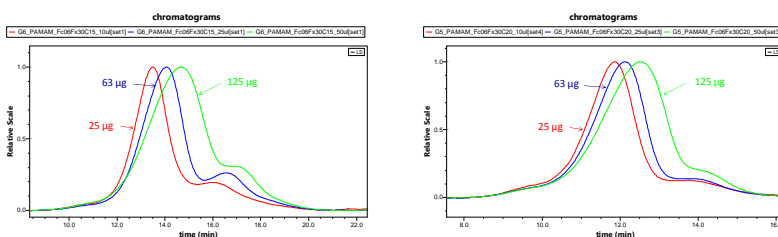
Fractograms of G4-G7 PAMAM



Fractogram Overlay of G4-G7



Variation of Injection Amounts for G5 and G6



Summary

- PAMAM Dendrimers and their fragments and aggregates can be readily separated by FFF.
- Molar mass distributions by FFF-MALS of a G4-G7 amine terminated PAMAM series shows increasing amount of fragments and aggregates for higher dendrimers.
- Higher generations have a lower molar mass than expected, probably due to defects (incomplete polymerization reaction).
- Hydrodynamic radii measured by are close to their expected values, batch DLS reveals the presence of aggregates and association for higher species.