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Fluorescence Analysis Of PAMAM Dendrimers

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ABSTRACT:

Despite their lack of conjugation, polyamidoamine (PAMAM) dendrimers are intrinsically fluorescent, and this fluorescent is somewhat manipulable through altering the dendrimer's solvent. This level of fluorescence is dependent on a number of the dendrimer's qualities: the pH of the solvent, the mass percent concentration of the dendrimer in solvent, and the generation size of the dendrimer. In past studies, fluorescent intensity increased as generation size increased and pH decreased. Thus, this research project will attempt to replicate those results as well as further analyze the efficiency of fluorescence of PAMAM dendrimer in comparison to fluorescein.

INTRODUCTION:

Light, as both a wave and a particle, affects nearly all facets of scientific study. Light can be either induce chemical processes or be emitted as a result, and the understanding of light has yet to be fully illuminated. Some substance emit light through chemical interaction alone in the form of luminescence, but the focus of this research is to study the means of light emission via light absorption, or in other words, fluorescence.

Years of research has elucidated the mechanisms behind typical fluorescence: singlet electrons in their ground states absorb the energy of an incoming photon, become excited into a higher energy orbital, and ultimately emit light as the electron recedes back into the ground state (Lavis & Raines, 2008). This mechanism is typically seen in conjugated molecules, chemical structures containing many alternating single and double bonds (see **Figure 1.**). Inherent in the conjugation of the molecule is rigidity around the double bonds. As photons of light impact the atomic structure, energy cannot be dissipated through the mechanical motion of rotating bonds. Because of the presence of the many double bonds, the energy of the photon is thus absorbed by the ground state electrons within the molecule and emitted as a lower energy light.

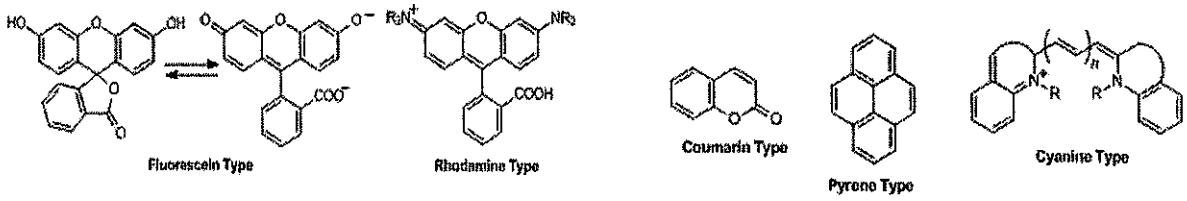

Figure 1. The chemical structure of conjugated fluorescent molecules.

The way this property is analyzed is through use of fluorescence spectroscopy. In simple terms, a quartz cuvette of fluoreseent solution is excited by a certain range of wavelength of light and the resultant emissions are recorded by a fluorescence spectrophotometer. The difference of wavelengths between the peak intensity of light exciting the molecule and the peak

intensity of light emitted from the molecule is known as the Stoke Shift and marks how efficient a molecule can transfer light absorbed into light emitted (see **Figure 2.**). The peak output can be affected by the concentration of the solution, and an important phenomenon to understand is the quenching affect. Quenching occurs when the presence of excess fluorescent molecules interferes with emissions of other fluorescent molecules, thus diminishing the overall emission of the solution.

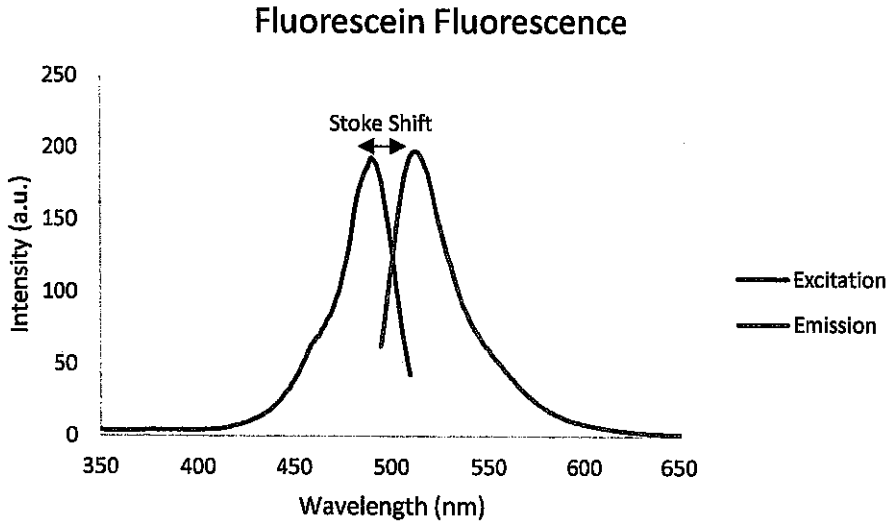

Figure 2. Fluorescence spectra of fluorescein.

The capacity for fluorescence in conjugated molecules has been researched extensively; fluorescence of conjugated molecules is widely understood. However, recent studies have shown that other molecules, more specifically, dendrimers, possess fluorescent properties yet lack conjugation. At first glance, the makeup of a typical dendrimer differs greatly from the molecules of **Figure 1**. Composed of branching monomers about a central core structure, these molecules are mostly single bonds with few double bonds, if any, scattered about their functional groups (see **Figure 3.**). This lack of conjugation has led to the conclusion that intense steric strain, the restriction of bond rotation due to electron repulsion, can mimic the rigidity of double

bond rigidity.

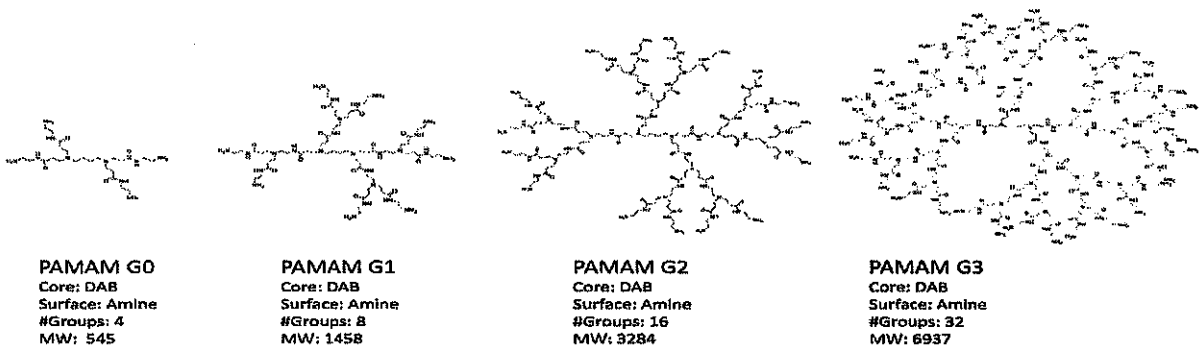

Figure 3. Different generation sizes of polyamidoamine (PAMAM) dendrimer. Dendrimers are nomenclated by Generation-Core-Surface.

The polyamidoamine (PAMAM) dendrimer has shown to be a fluorescent molecule that fluoresces better in generation 4 compared to generation 2 and at a low pH solution (Wang & Imae, 2004). Another study concluded that PAMAM dendrimers are also susceptible to quenching, and this quenching is more apparent when the dendrimers act as electron donors (Koley & Ghosh, 2016). Based on these results, the fluorescence of dendrimers seems to support the notion of fluorescence through steric strain; higher generations of dendrimer fluoresce better and dendrimers lacking electrons are quenched. With this in mind, the goal of this project will be to expand on this knowledge by looking at different generation sizes in different concentrations and pH solutions. Through these comparisons, we will also seek to quantify the efficiency of PAMAM dendrimer fluorescence in comparison to the conjugated molecule fluorescein using the quantum fluorescence yield equation (Allen, 2010).

The practical applications of understanding this kind of fluorescence comes in utilizing the different structure of dendrimer in comparison to typical fluorescing molecules. Dendrimer have multiple branches of varying functional groups, and each of these groups is reactive and can

potentially interact with other molecules. Dendrimers thus have the potential to bind to other molecules in a way fluorescein and other conjugated molecules cannot. If the fluorescence of dendrimers can be efficiently maximized to a comparable level to fluorescein, then the possible use of dendrimers as biomolecular tags can allow for fluorescence of other molecules through these dendrimers.

METHODOLOGY:

Solution Preparation

The three types of PAMAM dendrimer used in this experiment, G3-HEX-NH_2, G4-DAB-NH_2, and G5-DAB-NH_2, were initially synthesized by the Andrews ChemServices in the Department of Chemistry and Biochemistry[1]. Each dendrimer, initially in a 10% weight in methanol solution, was first air dried to blow off the methanol. Then the solution was subsequently vacuum dried to ensure the maximum amount of methanol had been evaporated. This left a viscous, pure dendrimer material that would be utilized in preparing specific solutions for each generation of dendrimer.

For each generation, the dendrimer solute was dissolved in three differing pH solvents: deionized water for pH 7, a sodium acetate/acetic acid solution for pH 4, and a potassium chloride/hydrochloric acid buffer for pH 2. In order to establish a pH 4 solution, 84.7 ml of 1 M acetic acid was mixed with 15.3 ml of 1 M aqueous sodium acetate. For the pH 2 solution, 50 ml of 1 M aqueous potassium chloride was mixed with 13 ml of 1 M hydrochloric acid. Each solution evaluated with pH paper to ensure pH accuracy. With each pH solvent, a 1%, 0.1%, and 0.01% weight solution was prepared with each generation. Each solution was analyzed and stored at approximately room temperature, about 20° C.

Fluorescence Analysis

Using the Cary Eclipse Fluorescence Spectrophotometer, the excitation and emission data for each dendrimer was analyzed in a quartz cuvette. Each dendrimer was excited at 420 nm, and the excitation spectra showed two excitation peaks: one in the 240-260 nm range and the other in

[1] Although each the G3 dendrimer has a different core molecule from the G4 and G5 dendrimers, this variance should not affect the overall results. The difference in cores leads to a negligible molecular weight difference in comparison to the overall molecular weight. The reason for the discrepancy is the limited resources of Andrews ChemServies.

the 340-370 nm range. The emission spectra were then analyzed by exciting the dendrimer at their respective excitation peaks to record the levels of emission. Each spectrum was exported into Microsoft Excel in order to view the data as functions of intensity (in arbitrary units a.u. in the y-axis) and wavelength (in nanometers in the x-axis).

Fluorescein Comparison

Once the spectra of all the dendrimer solutions were recorded, a fluorescein equivalent was prepared to match the fluorescence of 1 wt% G3-DAB-NH_2 in deionized water. Decreasing concentrations of fluorescein salt in deionized water were prepared and analyzed under the Cary Eclipse Fluorescence Spectrophotometer until the excitation peak of the fluorescein matched the excitation peak of the 1% weight G3 dendrimer.

Using this concentration in mind, the fluorescence quantum yield was then calculated using the following formula:

$$Q = Q_r \left(\frac{I}{I_r}\right)\left(\frac{OD_r}{OD}\right)\left(\frac{n^2}{n_r^2}\right) \qquad \textit{Single Point Fluorescent Quantum Yield Calculation}$$

Q is fluorescence quantum yield, I is the integrated fluorescence intensity, n is the refractive index of solvent, and OD is the optical density (absorption). The subscript "r" refers to the reference fluorophore of known quantum yield which is fluorescein for this work being proposed. Because the two solutes of dendrimer and fluorescein were dissolved in deionized water, the indices of solvent were identical and thus factor out to 1. The known value of the fluorescence quantum yield of fluorescein in water, or Q_r is 0.79. The data points of the fluorescein spectra with the dendrimer spectra will thus give the fluorescence quantum yield of the dendrimer.

Results

The G3-HEX-NH_2 pH 7, 4, and 2 solutions showed approximately proportional decreases of fluorescence as concentration decreased by factors of 10. In the 1% weight solution, the 255-nm excitation peak exhibited less intensity than the 335-nm peak, but as the concentration decreased, the 355-nm peak diminished at a greater rate than the 255-nm peak. Each solution at the 0.01% weight showed a 255-nm peak that was greater than the 355-nm peak. The emissions of each peak also followed this trend with their respective excitations. The conditions in which G3 dendrimer fluoresced the greatest were in 1% weight pH 4 solution. The Stoke Shift variance was relatively consistent (see **Figure 4, Tables 1-3**).

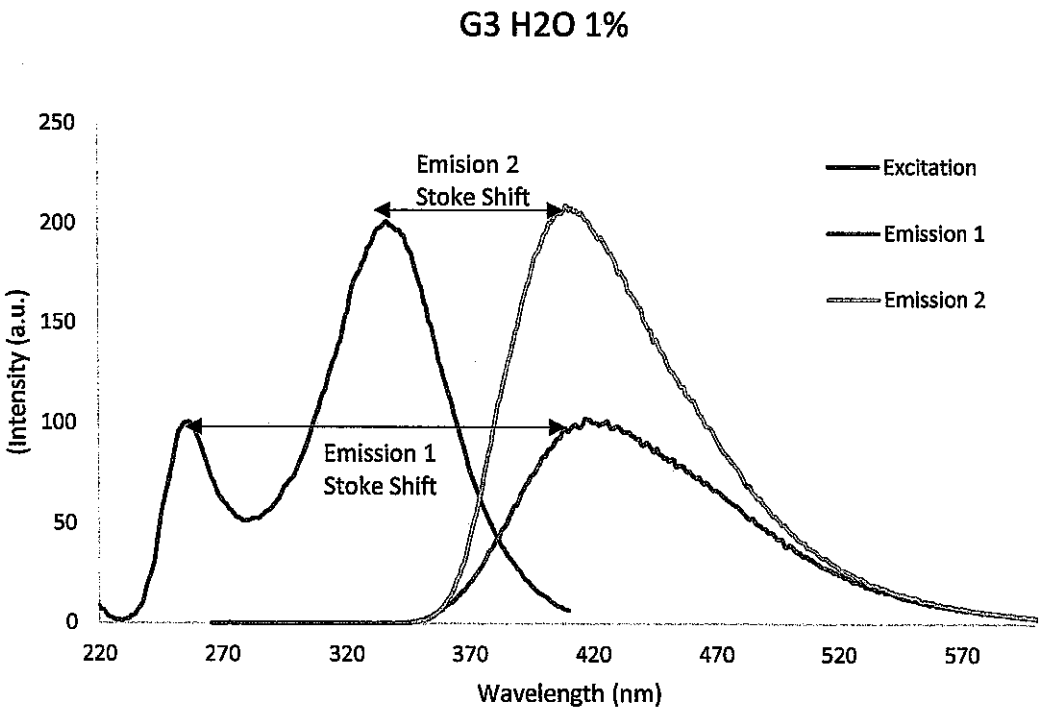

Figure 4. Excitation spectra of G3 1% weight solutions of pH 7. Emission 1 refers to the emission of the 240-260 nm excitation of PAMAM dendrimer, and Emission 2 refers to the

emission of the 340-370 nm range of excitation. The respective Stoke Shifts measure the distance between the peaks of excitation and emission.

Concentration	Emission 1 Peak	E-1 Stoke Shift	Emission 2	E-2 Stoke Shift
1%	100 a.u.	161 nm	209 a.u.	79 nm
0.1%	19 a.u.	168 nm	23 a.u.	76 nm
0.01%	2.1 a.u.	172 nm	2.8 a.u.	113 nm

Table 1. Fluorescence trends of G3 dendrimer in pH 7 solution.

Concentration	Emission 1 Peak	E-1 Stoke Shift	Emission 2	E-2 Stoke Shift
1%	250 a.u.	189 nm	310 a.u.	69 nm
0.1%	32 a.u.	169 nm	36 a.u.	72 nm
0.01%	16 a.u.	173 nm	6.8 a.u.	85 nm

Table 2. Fluorescence trends of G3 dendrimer in pH 4 solution.

Concentration	Emission 1 Peak	E-1 Stoke Shift	Emission 2	E-2 Stoke Shift
1%	245 a.u.	172 nm	238 a.u.	73 nm
0.1%	19 a.u.	158 nm	23 a.u.	67 nm
0.01%	15 a.u.	147 nm	1.7 a.u.	78 nm

Table 3. Fluorescence trends of G3 dendrimer in pH 2 solution.

The G4-DAB-NH_2 dendrimer showed a similar trend of proportional decrease of the 340-nm emission while the 240-nm decreased at a slower rate. However, as pH decreased, the fluorescence of G4 dendrimer increased, with the highest peak of emission of 1% weight in pH 4 solution. Stoke Shift variance occurred more readily in the lower concentration solutions for the 320-nm emission (see **Tables 4-6**).

Concentration	Emission 1 Peak	E-1 Stoke Shift	Emission 2	E-2 Stoke Shift
1%	100 a.u.	154 nm	156 a.u.	81 nm
0.1%	19 a.u.	172 nm	22 a.u.	92 nm
0.01%	3.7 a.u.	189 nm	1.6 a.u.	114 nm

Table 4. Fluorescence trends of G4 dendrimer in pH 7 solution.

Concentration	Emission 1 Peak	E-1 Stoke Shift	Emission 2	E-2 Stoke Shift
1%	145 a.u.	171 nm	255 a.u.	96 nm
0.1%	41 a.u.	187 nm	31 a.u.	95 nm
0.01%	24 a.u.	154 nm	7.8 a.u.	100 nm

Table 5. Fluorescence trends of G4 dendrimer in pH 4 solution.

Concentration	Emission 1 Peak	E-1 Stoke Shift	Emission 2	E-2 Stoke Shift
1%	108 a.u.	188 nm	343 a.u.	69 nm
0.1%	21 a.u.	187 nm	37 a.u.	95 nm
0.01%	6.8 a.u.	173 nm	4.7 a.u.	106 nm

Table 6. Fluorescence trends of G4 dendrimer in pH 2 solution.

The G5-DAB-NH_2 dendrimer followed a trend similar to the G3 dendrimer in terms of the effect of pH on fluorescence. The solution in which fluorescence was the greatest was in the 1% weight pH 4 solution, though the pH 2 solutions showed greater fluorescence than the pH 7 solutions. Once again, the 240-nm emissions decreased at a lesser rate than the 340-nm emissions as concentration decreased. The Stoke Shifts also showed little variance (see **Tables 7-9**).

Concentration	Emission 1 Peak	E-1 Stoke Shift	Emission 2	E-2 Stoke Shift
1%	119 a.u.	158 nm	222 a.u.	81 nm
0.1%	24 a.u.	168 nm	25 a.u.	84 nm
0.01%	3.1 a.u.	181 nm	1.6 a.u.	86 nm

Table 7. Fluorescence trends of G5 dendrimer in pH 7 solution.

Concentration	Emission 1 Peak	E-1 Stoke Shift	Emission 2	E-2 Stoke Shift
1%	363 a.u.	196 nm	546 a.u.	69 nm
0.1%	94 a.u.	180 nm	64 a.u.	85 nm
0.01%	65 a.u.	161 nm	17 a.u.	105 nm

Table 8. Fluorescence trends of G5 dendrimer in pH 4 solution.

Concentration	Emission 1 Peak	E-1 Stoke Shift	Emission 2	E-2 Stoke Shift
1%	139 a.u.	188 nm	336 a.u.	72 nm
0.1%	29 a.u.	142 nm	29 a.u.	99 nm
0.01%	14 a.u.	149 nm	7.5 a.u.	102 nm

Table 9. Fluorescence trends of G5 dendrimer in pH 2 solution.

The Fluorescein equivalence to G3 came out to be a concentration of about 4.440e-5% weight in deionized water. In comparison to the actual concentration of 0.9953% G3 dendrimer, the quantum fluorescence yield for G3 dendrimer was calculated to be approximately 1.18e-5. The Stoke Shift for fluorescein was observed to be about 21 nm.

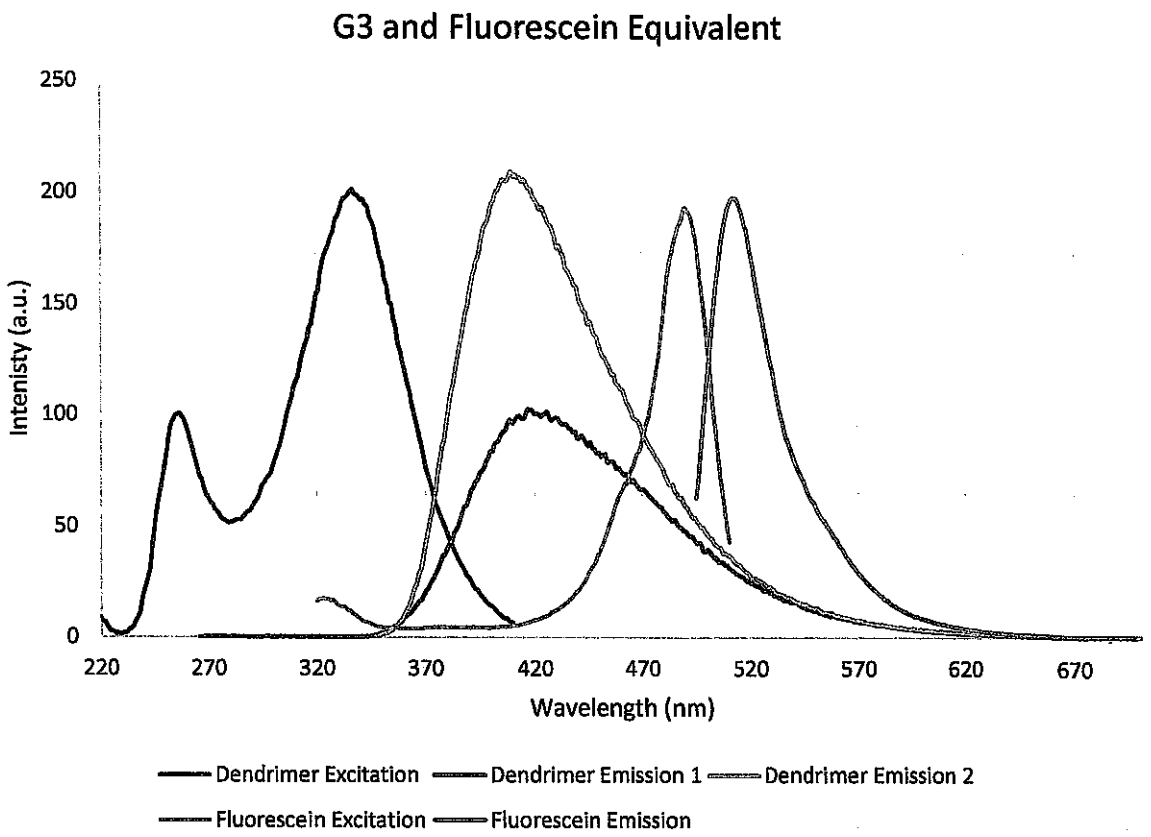

Figure 5. Comparison of excitation and emission spectra of G3 PAMAM dendrimer with fluorescein.

Discussion

Across all of the emissions with each generation of dendrimer, as the generation increased, the peak emission also increased. This correlates with the knowledge that greater generations of dendrimer exhibit higher amounts of steric strain, and thus become more rigid and fluoresce better. However, this trend did not appear to be consistent when factoring the effect of pH. In the pH 7 and pH 4 solution, G4 dendrimer had overall the lowest peak fluorescence, while the pH 2 solutions displayed the expected trend of G3 fluorescing the least and G5 fluorescing the most. This deviates from Larson & Tucker's findings on the effect of generation size on

dendrimer fluorescence. Using carboxylate-terminated PAMAM dendrimers ranging from generations 2.5 to 7.5 in methanol, a successive increase in fluorescence was observed as generation increased (Larson & Tucker, 680-681). Another unexpected trend showed that G3 and G5 dendrimer fluoresced the best in pH 4 solution, while G4 dendrimer fluoresced the best in pH 2 solution. The observed peak of G4 in pH 2 correlates with Wang & Imae's observations, as the study showed that G4-NH_2 terminated PAMAM dendrimer increasingly fluoresced as the pH decreased (13204).

The discrepancy in results may be due to the interactions of the surface functional groups with the solution medium. As the polyamidoamine contains both an amide and amine group, there are two groups that become protonated and, as a result, affect the molecular interactions between the branches of dendrimer. The ratio of polyamidoamine branches that differs across generations may lead to a different level of protonation in a given pH, and thus the functional groups of the branches seem to affect the fluorescent capacity of PAMAM dendrimer. A future study could focus on the effect different types of functional groups on the terminal end of a dendrimer and their relative abundance in each generation on fluorescence in pH.

In its current form, G3 PAMAM dendrimer proves to be much less efficient in fluorescing than the conjugated molecule, fluorescein. Much of the data of the two molecules show the different capacities of each to fluoresce. Fluorescein has a fluorescence quantum yield of 0.79 in deionized water, while the yield of G3 PAMAM dendrimer in a concentration of equivalent fluorescence was 1.18e-5. This shows that fluorescent yield of PAMAM dendrimer is more than 10,000 less efficient than fluorescein. Comparing the concentrations, a 4.44e-5% weight concentration of fluorescein is able to fluoresce at an equivalent intensity than a 1% weight of G3 dendrimer. Also observing the Stoke Shifts shows how much more efficient

fluorescein fluoresces. The difference in wavelengths between the excitation and emission of fluorescein was 21 nm while both the Stoke Shifts of PAMAM dendrimer were well above 60 nm.

But although PAMAM dendrimers do not fluoresce as efficiently as fluorescein, they undeniably possess the capacity for fluorescence. Generally, these molecules fluoresce better in acidic environments with higher generations, supporting the idea that steric strain mimics double bond rigidity. Continuations of this study could expand the solution concentrations, pH range, and comparisons to other fluorescent molecules. In addition to these expansions, different generations of PAMAM dendrimer along with other dendrimers with different functional group terminals could expand the understanding of the phenomenon of dendrimer fluorescence.

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