

INVESTIGATION INTO INTERMOLECULAR INTERACTIONS OF $Gd_3N@C_{80}(OH)_n$
USING FLUORESCENCE SPECTROSCOPY

by

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INTRODUCTION

The aim of this experiment was to investigate the intermolecular interactions of the endohedral metallofullerene complex $Gd_3N@C_{80}(OH)_n$ in water and various metal cation solutions. These interactions were observed using a methodology that is particularly sensitive to environmental changes surrounding the probe molecule: fluorescence spectroscopy. Changes in the intensity (I) of the fluorescence peak were monitored to indicate and characterize the nature of these intermolecular interactions. Certain types of interactions will enhance fluorescence while other modes will reduce fluorescence intensity. The results of this investigation are presented within this report.

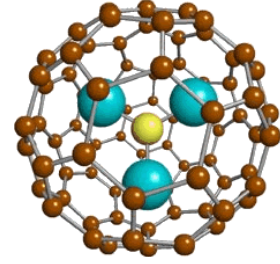


Figure 1:
 $Gd_3N@C_{80}(OH)_n$

THEORY

A. FLUORESCENCE INTENSITY

In this experiment, intermolecular interactions between the probe molecule and various solutions were investigated utilizing fluorescence spectroscopy. Spectra can be a valuable tool to elucidate interactions between solute and solvent molecules because, in many cases, the interactions cause significant changes in the characteristics of the observed spectra. These interactions are observed as increasing or decreasing the intensity of the fluorescence peak. In this experiment, changes in fluorescence intensity were used to characterize interactions of the probe molecule in solutions of water and various metal ion species.

The 3-D Gaussian excitation intensity distribution is given by Equation 1, where z is the distance from the focal plane along the optical axis and r is the distance to the optical axis. ω_1 and ω_2 designate the distances in the radial and axial direction from the center of the volume element at which the fluorescence intensity has decreased by a factor of e^2 .

$$\Phi(r, z) = \Phi_0 e^{-2r^2/\omega_1} e^{-2z^2/\omega_2} \quad (1)$$

Equation 2 indicates the normalized intensity autocorrelation function in the case that the only dynamic process causing fluctuations in fluorescence intensity is the diffusion of the fluorophores in and out of the sample volume element. N indicates the number of molecules in the sample volume element, D indicates the translational diffusion coefficient and $\langle I \rangle$ indicates the detected mean intensity.

$$G_n(\tau) = \lim_{T \rightarrow \infty} \frac{1}{T} \int_0^T \frac{I(t+\tau)I(t)}{\langle I \rangle^2} dt = \frac{1}{N} \left(\frac{1}{1+4\frac{D\tau}{\omega_1^2}} \right) \left(\frac{1}{1+\frac{4D\tau}{\omega_2^2}} \right)^{1/2} \quad (2)$$

The laser beam profile and the projected dimensions of the pinhole will be used to determine the detected fluorescence intensity profile. The total detected fluorescence intensity $I(t)$ is given by Equation 3. $i(r, z, t)$ indicates the detected fluorescence per unit

volume and σ_{exc} denotes the excitation cross section of the fluorophore. The $CEF(r,z)$ parameter is the collection efficiency function, $c(r,z,t)$ indicates the concentration of the fluorophores and $\phi(r,z)$ is the excitation intensity of the laser. The symbol q denotes the quantum efficiency of the detector, the fluorescence quantum yield of the fluorophore and the attenuation of the fluorescence in the passage from the sample droplet to the detector.

$$I(t) = \iiint i(r, z, t) dV = \iiint q CEF(r, z) \sigma_{exc} \Phi(r, z) \times c(r, z, t) dV \quad (3)$$

In the derivation of Equation 1, it is assumed that saturation of the fluorophores has not been reached and that the distribution of $i(r,z,t)$ is Gaussian and linear to the function $\phi(r,z)$. In order to maximize FCS measurements, the tradeoff between increasing triplet state population and saturation when using high excitation intensities must be considered. (Widengrin et al, 1995)

B. INTERMOLECULAR INTERACTIONS AND FLUORESCENCE INTENSITY

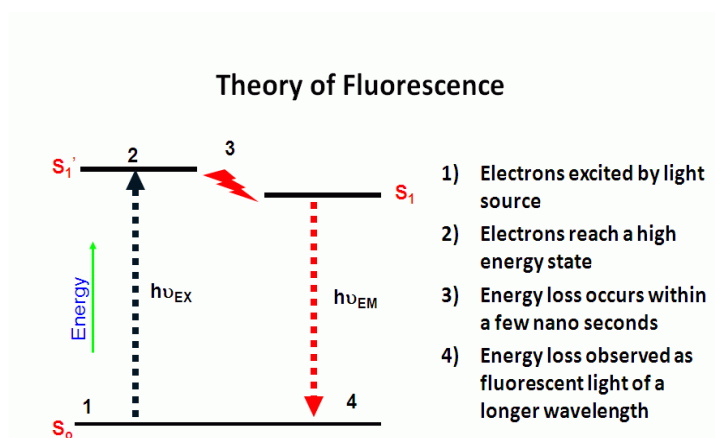


Figure 2: A graphical representation of the process of fluorescence. Intermolecular interactions, of interest in this experiment, occur in step 3 and are visible as changes in fluorescence intensity in step 4.

Fluorescence intensity is affected by intermolecular interactions by a relatively simple scheme, as portrayed in Figure 2. The probe molecule absorbs radiation at $\lambda=650$ nm which excites electrons from a ground state to a particular excited state. At this point, the probe molecule interacts with its environment and particles surrounding, which can increase or decrease the energy of the electrons. In the case of this experiment, the probe molecule's environment consists of either water or metal cations. The excited probe molecule then gives off a characteristic energy that is observed as the fluorescence intensity of the molecule. The primary intermolecular interactions observed in this study were hydrogen-bonding and metal-ligand charge transfer interactions.

A hydrogen bond is an electromagnetically attractive interaction based on a particularly strong dipole-dipole force between molecules in which Hydrogen is bound to a highly electronegative atom. In the case of this experiment, the probe molecule was introduced into a water solution and the hydroxides on the surface of the complex interacted with the water molecules in its environment. This interaction causes a change in electron density of the probe, donating it from the complex to the water molecules surrounding. This causes “quenching” of the fluorescence spectrum, a qualitative decrease in fluorescence intensity of the molecule (Zhang, 2013).

Metal-ligand charge transfer is an intermolecular interaction wherein electrons are transferred between a metal species and a ligand. In the case of this experiment, electrons are transferred between the metallofullerene complex and the metal ions, Zn^{2+} and Cu^{2+} , in solution. This interaction causes a distinctive increase in the electron density surrounding the complex. Subsequently, the fluorescence intensity of the molecule is increased significantly (Zhang, 2013).

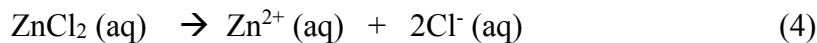
EXPERIMENTAL

A. Chemicals used.

- $Gd_3N@C_{80}(OH)_n$
- D_2O
- H_2O
- $ZnCl_2$
- $CuSO_4$

B. Solution Preparation.

1. $ZnCl_2$ and $CuSO_4$ solutions. $ZnCl_2$ and $CuSO_4$ solutions were prepared in D_2O with the same concentration as was used in the preparation of $Gd_3N@C_{80}(OH)_n$. The $ZnCl_2$ and $CuSO_4$ solutions generate the cations as illustrated in the two chemical equations given below:



2. Fluorescence Spectra of $Gd_3N@C_{80}(OH)_n$ in D_2O . A solution of the probe molecule was made using 10 uL of $Gd_3N@C_{80}(OH)_n$ and 0.75 mL of D_2O . A fluorescence spectrum of this solution was taken with an excitation of 350 nm. A distinctive peak was observed in the region of ~690-710 nm range. 10 uL of water was added to this solution and a fluorescence spectrum was taken, scanning in the 650-750 nm range. This process was repeated for solutions with 30, 60, 80 and 100 uL of water.
3. Zn^{2+} and Cu^{2+} solutions with $Gd_3N@C_{80}(OH)_n$. A solution of the probe molecule was made using 10 uL of $Gd_3N@C_{80}(OH)_n$ and 0.75 mL of D_2O . In

order to observe the characteristics of the molecule interacting with metal ions, solutions of Zinc Chloride ($ZnCl_2$) and Copper Sulfate ($CuSO_4$) were prepared. 5 mg of Zinc Chloride were dissolved in 1.5 mL D_2O and similarly, 5.2 mg of Copper Sulfate were dissolved in 1.5 mL D_2O .

4. Fluorescence Spectra of $Gd_3N@C_{80}(OH)_n$ with Zn^{2+} and Cu^{2+} . 10 μ L of Zinc Chloride was added to the probe molecule solution and a fluorescence spectrum was taken with excitation at 350, scanning from 650-750 nm. It was observed that the intensity of the peak was enhanced significantly upon addition of Zinc. This procedure was repeated for 30, 60 and 80 μ L of Zinc. At this point it was clear that the detector had been saturated with signal. Subsequently, 10 μ L of Copper Sulfate were added to the probe molecular and a fluorescence spectrum was taken. It was observed that the addition of Copper increased the peak intensity significantly. This procedure was repeated for 30, 60, 80 and 100 μ L of Copper Sulfate solution.

DATA

TABLE 1: Results of fluorescence Spectra of $Gd_3N@C_{80}(OH)_n$ in H_2O

Vol. of H_2O (uL)	λ (nm)	Intensity (a.u.)
0 uL	697	10,739
10 uL	698	8,617
30 uL	698	7,489
60 uL	698	6,879
80 uL	698	6,612
100 uL	697	6,138

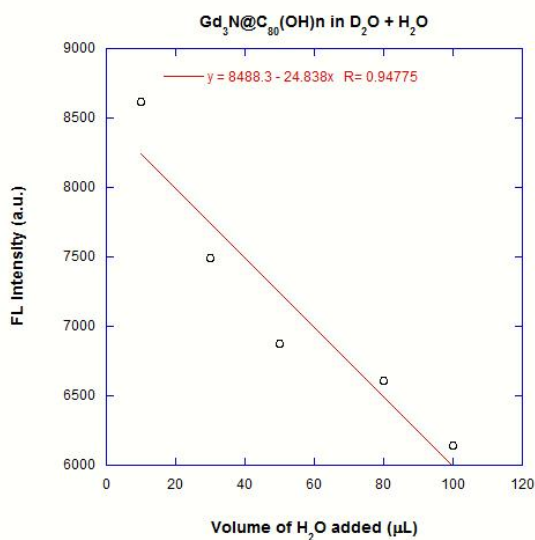


Figure 2: The graph of the volume of water added plotted against the fluorescence intensity (a.u.) of the probe molecule.

Above, the results of the series of fluorescence spectra of $Gd_3N@C_{80}(OH)_n$ in increasing amounts of water are delineated. A clear negative trend can be observed between the volume of water added and the intensity of fluorescence of the probe molecule. This negative trend in the data was fitted to a line of equation: $y = 8488.3 - 24.838x$ with an “R value” of 0.94775 for this relationship.

TABLE 2: Results of fluorescence spectra of $Gd_3N@C_{80}(OH)_n$ in $ZnCl_2$

Vol. of Zn^{2+}	λ (nm)	Intensity (a.u.)
10 uL	698	10,176
30 uL	697	22,897
60 uL	698	22,992
80 uL	698	22,725

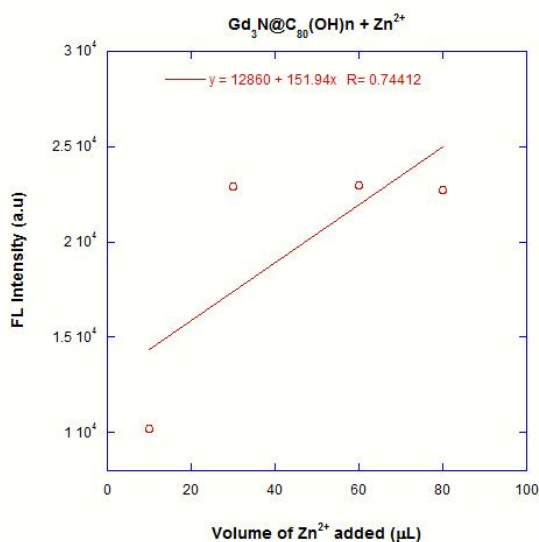


Figure 3: The graph of the volume of Zinc Chloride added plotted against the Fluorescence intensity (a.u.) of the probe molecule.

The results of the series of fluorescence spectra of the endohedral metallofullerene complex $Gd_3N@C_{80}(OH)_n$ in increasing amounts of zinc chloride are delineated above. The results of this relationship are not so clear, unfortunately. It is clear that the presence of the Zinc ion in solution enhances the intensity of the fluorescence of the probe molecule, that is to say that a positive correlation exists between the two variables. However, it is clear that the detector was saturated with signal too quickly to be able to elucidate any useful quantitative relationship between them. The relationship between volume of Zn added and fluorescence intensity determined from this experiment was $y=12860+151.94x$ with an R value of 0.74412.

TABLE 3: Results of fluorescence spectra of $Gd_3N@C_{80}(OH)_n$ in $CuSO_4$

Vol. of $CuSO_4$ (uL)	λ (nm)	Intensity (a.u.)
10 uL	697	16,890
30 uL	698	24,863
60 uL	698	22,992
80 uL	698	24,288
100 uL	698	21,230

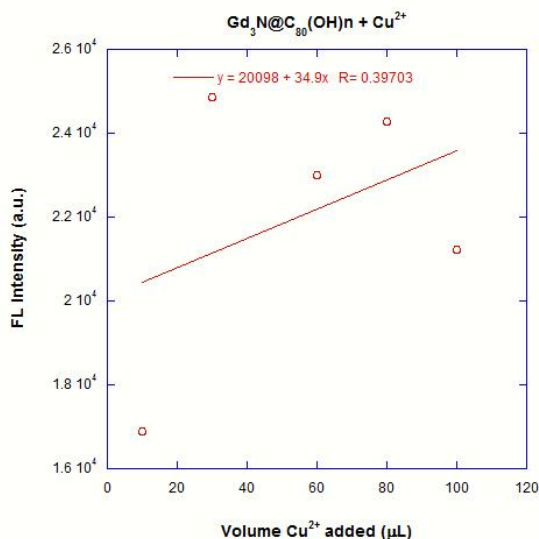


Figure 4: The graph of the volume of copper sulfate added plotted against the fluorescence intensity (a.u.) of the probe molecule.

Above, the results of the series of fluorescence spectra of the probe molecule, $Gd_3N@C_{80}(OH)_n$, in increasing amount of copper sulfate are outlined. The results of this study of the intermolecular interactions are also a little ambiguous. While a clear, positive trend does exist between the volume of copper added and the fluorescence intensity, the relationship inferred from these data is not reliable at all. It has been hypothesized that the detector was saturated with signal and thus, the nature of the relationship cannot be inferred. The results of this trial are thus, inconclusive.

RESULTS AND DISCUSSION

When water was introduced to the probe molecule it was found that the fluorescence intensity of the solution was decreased significantly. When a series of increasing amounts of water was introduced into the system, the intensity changed in a negative trend giving the quantitative equation $y = 8488.3 + 24.838x$ with an R value of 0.94775 for the relationship. This decrease in fluorescence intensity is called “quenching”. It is hypothesized that this quenching is due to hydrogen bonding intermolecular interactions between the probe molecule and the water molecules in solution. The reduction in fluorescence intensity results from an interaction between the water molecules and the hydroxyl groups (-OH) on the probe molecule which removes electron density from the probe molecule and hence reduces the fluorescence intensity.

When Zinc (Zn^{2+}) was introduced into the probe molecule solution, it was found that the intensity of the fluorescence peak increased significantly and when a series of increasing amounts were added, the increase in intensity formed a distinct positive trend. This relationship is given by the quantitative equation $y=12860+151.94x$ with an R value of 0.7445. This significant increase and positive relationship between the amount of Zn added and the fluorescence intensity can be attributed to an intermolecular interaction between the probe molecule and the metal ion called metal-ligand charge transfer. The Zinc data indicates that this type of intermolecular interaction “pumps” electrons back into the probe molecule via the mechanism known as “ligand to probe electron transfer.” Though, it seems that the detector quickly became saturated with signal, the interaction is most definitely there. Further research in the range of adding 0-20 microliters of solution would make the relationship much clear.

When Copper (Cu^{2+}) was added to the probe solution, it was found that the intensity of the fluorescence peak increased significantly. Though, when increasing amounts of the solution were added, the relationship became a little unclear and difficult to discern, increasing at certain points (from 10-30 uL) and decreasing at others (30-60 uL). Thus, the intermolecular interaction is definitely there, however, the exact nature of it is, as of now, a little uncertain. Two hypotheses have been offered as to why this relationship may have occurred. First, that the detector was saturated with signal and could not show the true nature of the relationship. Second that some kind of rapid equilibrium exists between the probe molecule and the metal ions in solution. Further research into the nature of this interaction may shed some light on these issues. Studies using complexing agents such as EDTA have been suggested to study this issue.

References Used

1. Yamashita, Y.; Jaffe, R., *Environ. Sci. Technol.*, 2008, 42, 7374.
2. Zhang, G.; Wen, Y., *J. Fluoresc.*, 2013, 23, 1053-1063.
3. Ghatak, C.; Rao, V., *J. Phys. Chem.*, 2012, 116, 3369-3379.
4. Matson, M.; Carlsson, N., *Langmuir*, 2012, 28, 10808-10817.
5. Reddy, M.; Taha, M., *Polymer*, 2013, 54, 791-797.
6. Widengren, J.; Mets, U., *J. Phys. Chem.*, 1995, 99, 13368-13379.