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# Oxidization and aggregation of first generation poly(amidoamine) with an enhanced fluorescent performance

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ABSTRACT

Organic amines are apt to be oxidized in natural environment. Therefore, preparation of fluorescent materials derived from organic amines should think of the role of oxidized amines. However, this is neglected according to some reports. Herein, first generation poly(amidoamine) (**PAMAM 1.0G**) dendrimers with inherent weak photoluminescence was oxidized by ambient oxygen under heating of 180 °C for 60 min which brought an obvious enhancement of fluorescent quantum yield (QY). The oxidation was proved by both infrared and $^{1}$H nuclear magnetic resonance. Image of transmission electron microscope (TEM) also revealed the aggregation of **PAMAM 1.0G** after they were suffered from the lasting heating. The enhancement of QY was also observed when the mixture of aqueous solution of **PAMAM 1.0G** and citric acid were disposed under the same conditions. And the QY reached as high as 64.40%, which is much higher than that of the fluorescent materials derived from sole citric acid. TEM image also exhibited the aggregation of the product. The carboxyl of the aggregated particle could be activated by N-hydroxysuccinimide and could be bonded with bovine serum albumin realizing the fluorescent labeling of BSA finally, which was demonstrated by the image of gel electrophoresis.

## 1. Introduction

Organic amines are utilized extensively in making fluorescent materials especially in preparation of fluorescent carbon quantum dots (CQDs). The doped amines contributed a lot for the enhancement of fluorescent features of the CQDs. Nitrogen-doped CQDs were usually obtained by heating the mixture of carbohydrate and organic amine such as ethylenediamine [1], diethylenetriamine [2], linear-structured polyethyleneimine [3], ethanolamine [4], ethylenediamine-tetraacetic acid [5] and branched polyethylenimine [6], etc. The produced CQDs performed a higher fluorescent quantum yield (QY) compared with that of sole carbohydrate. However, amino groups including tertiary amine are inclined to be oxidized by the surrounding oxygen, which will be beneficial for the enhancement of fluorescence. Imae and his coauthors reported that the fluorescent intensity of the solution of the forth generation of poly(amido amine) (**PAMAM**) increased a lot when exposed to air for 25 days [7]. Hence, when the fluorescent material derived from organic amine was planed to be prepared the oxidation of amine should be considered carefully. During the generation of nitrogen-doped CQDs amines might be oxidized by the surrounding oxygen. Perhaps the fluorescent performance might be contributed mainly by the oxidation of amine. These oxidized amines were hard to be removed completely in purification of the product due to their large molecular weight [3,6,8,9]. Hence the fluorescence of the CQDs should be attributed partly by the oxidized amines. Actually, some organic amines are prone to be oxidized under certain conditions such as aniline. Similarly, some fine chemicals such as dimethyl dodecyl amine oxide were prepared by oxidation of the corresponding amine [10]. Unfortunately this was omitted in discussing the chemical structure and the cause of QY enhancement of the nitrogen-CQDs [2–6,8,9,11].

Since **PAMAM** does not possess conventional chromophores such as some conjugated species in the molecule the fluorescence phenomenon of oxidized **PAMAM** aroused extensive interests [12,13]. Bard found that OH-terminated **PAMAM** could be oxidized by ammonium peroxydisulfate leading to photoluminescence with a high QY [12]. Imae reported that the fluorescent intensity of **PAMAM 4.0G** depended on temperature and its concentration [14]. And the higher temperature and concentration brought a more intensive fluorescent intensity. Additionally, the decreased pH value also helped the increase of the fluorescent intensity. This might be ascribed to the molecular rigidity of **PAMAM** after the tertiary amine combined with protons [13]. Summarily, oxidation and rigidity of **PAMAM** induced the enhanced photoluminescence.

Herein, we would demonstrate the oxidation and aggregation of **PAMAM 1.0G** which brought the obvious enhanced fluorescent performance when the aqueous solution was suffered from a lasting

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heating until the solvent water was removed completely. The fluorescent performance could be enhanced successively when doped with citric acid and the product dissolved in aqueous medium very well. That promoted potential application of the **PAMAM** dendrimers in more areas that needed a higher fluorescent QY.

## 2. Experimental

### 2.1. Materials

The first to third generation of **PAMAM** dendrimers (**PAMAM 1.0G** ~ **3.0G**) were prepared by Michael addition of ethylenediamine and methyl acrylate and successively by amidation with ethylenediamine according to the literature [15]. The prepared **PAMAM** dendrimers were sealed and stored in refrigerator for more than one month (4 ~ 6 °C). Hydrogen peroxide ($H_2O_2$, 30%), citric acid monohydrate (99.5%), N-hydroxysuccinimide (98.0%), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC, 98.0%) and bovine serum albumin (BSA, ~66 kD) were purchased from Aladdin company (Shanghai, China). All reagents were used as received and the double-distilled water was used through all the experiments.

### 2.2. Heating treatment on aqueous solution of the PAMAM dendrimers

Aqueous solution of **PAMAM 1.0G** (5.5 mL, 54.7 mg $mL^{-1}$) was placed in an electric drying oven with forced convection at 180 °C for 60 min. On account of the high temperature the water component was evaporated gradually. Ultimately, yellow membrane on the bottle of the vessel was generated, which was collected and denoted with **OA-PAMAM 1.0G** thereafter. Fluorescent spectrum of **OA-PAMAM 1.0G** was measured and had a comparison with that of **PAMAM 1.0G**. Such a treatment was repeated under 60, 100, 140 °C respectively to inquire the effect of heating temperature on the fluorescent intensity of **PAMAM 1.0G** dendrimers.

In addition, **PAMAM 0.5G**, **1.5G**, **2.0G**, **2.5G** and **3.0G** dendrimers were disposed respectively with the same method (heating in the oven at 180 °C for 60 min) to investigate the changes of fluorescent QY before and after such a heating disposing.

### 2.3. Effect of water fraction in PAMAM 1.0G solution and oxidation of amino groups at the presence of $H_2O_2$ on the fluorescent performance

**PAMAM 1.0G** solutions of 2, 5, 10, 15 and 20 mL containing 416.2 mg **PAMAM 1.0G** (water fraction corresponds to 79.2%, 91.7%, 95.8%, 97.2% and 97.9% respectively) were placed in the drying oven simultaneously at 100 °C for 50 min. The samples of 2, 5, 10 mL were complete dried while the samples of 15 mL and 20 mL leaved about 1 and 3 mL respectively at the end of heating. Then a predetermined volume of water was added to obtain the solutions with the same total volume (8.00 mL). Such a solution of 5 μL was fetched and diluted with 3.00 mL water respectively to have a measurement of fluorescence spectrum. Their differences in fluorescent intensity were utilized to analyze the effect of the initial water fraction on fluorescent performance of **OA-PAMAM 1.0G** when they suffered from the lasting heating.

Aqueous solution of **PAMAM 1.0G** (5.5 mL, 54.7 mg $mL^{-1}$) at the presence of 1 mL $H_2O_2$ was treated in the oven at 180 °C for 60 min for inquiring the effect of intensive oxidation on the QY of **OA-PAMAM 1.0G**.

### 2.4. Heating treatment on the aqueous solution of PAMAM 1.0G at the present of citric acid

Weighted 151.7 mg citric acid and mixed with 5.5 mL aqueous solution of **PAMAM 1.0G** (54.7 mg $mL^{-1}$). The added citric acid was dissolved entirely and the solution became acidic (pH = 5.5). Then it was put into the drying oven at 180 °C and was heated for 60 min for obtaining the citric acid-modified **OA-PAMAM 1.0G** (denoted with **OA-PAMAM 1.0G-CA**).

### 2.5. Fluorescent labeling on BSA with OA-PAMAM 1.0G-CA

Fetched 2.2 mg **OA-PAMAM 1.0G-CA** and dissolved in 10 mL water followed with addition of 6.7 mg EDC. The reaction was kept at 30 °C for 30 min with a magnetic stirring. Then 3.8 mg NHS were involved in the reaction at 30 °C for 2 h. The product was purified with a dialysis tube (3000 kD) for 8 h for removing the impurities. During the dialysis the outside water was discarded and was refreshed three times. At last, 2 mL solutions from the dialysis tube were mixed with 2 mL BSA solutions (8.0 mg $mL^{-1}$ in phosphate buffered solution) and the mixture was kept at 25 °C for 6 h for realizing the chemical attachment of **OA-PAMAM 1.0G-CA** to BSA (**OA-PAMAM 1.0G-CA-BSA**). Then the mixture was loaded in a dialysis tube (7000 kD), which was placed in water for 8 h for farther purification. The outside solutions were removed and refreshed. Such a process was repeated for 3 times.

The obtained solution of **OA-PAMAM 1.0G-CA-BSA** in dialysis tube was employed in an experiment of sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Such solutions with 5 and 10 μL were dropped in the respective wells of the gel. The fluorescent labeling on BSA with **OA-PAMAM 1.0G-CA** was demonstrated by the photographs irradiated under an ultraviolet lamp. The gel was also stained with coomassie brilliant blue and was taken a picture in daylight.

### 2.6. Characterization techniques

The images of transmission electron microscopy (TEM, Tecnai G2 F20 S-TWIN FEI Company) revealed the morphology of aqueous **PAMAM 1.0G** solution before and after heating at 180 °C for 60 min and provided a proof of the aggregation. Infrared (IR, Nicolet 380, Thermo Nicolet Corporation) and $^1H$ nuclear magnetic resonance (NMR, BRUKER 300, TMS as an internal standard) spectra provided explicit evidences of oxidation of **PAMAM 1.0G** dendrimers during the lasting heating. Fluorescence spectra (LS 45, PerkinElmer) were estimated to judge the enhancement of fluorescent features of **OA-PAMAM 1.0G** and **OA-PAMAM 1.0G-CA**. Enhancement of the fluorescent intensity also provide a proof of oxidation of **PAMAM 1.0G**. During the measurement of fluorescent QY, quinine sulfate in 0.50 M $H_2SO_4$ solutions (QY = 55.00%) was chosen as a fluorescence standard to measure fluorescence QY. The QY was calculated according to the following formula.

$$QY_x = QY_Q \frac{Slope_x}{Slope_x} \frac{n_x^2}{n_Q^2}$$

where the subscript x and Q refer to the unknown sample and quinine sulfate respectively, Slope represents the slope of regressed line between integrated fluorescent intensity of the emission and absorbance, n is refractive index, $n_x/n_Q$ equals 1.0 approximately in this case. The fluorescent labeling on BSA with **OA-PAMAM 1.0G-CA** was demonstrated by the photographs of gel imaging (UVP GelMax Imager System, USA).

## 3. Results and discussion

### 3.1. QY enhancement of the heated PAMAM 1.0G

When the aqueous solution of **PAMAM 1.0G** dendrimers was placed in the drying oven at 180 °C for 60 min, the product, **OA-PAMAM 1.0G**, emit a stronger fluorescence, which can easily be judged from inset of Fig. 1(a) while the aqueous solution of **PAMAM 1.0G** performs weak photoluminescence. Fluorescence spectra of Fig. 1(a) disclose that the