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Study on Fluorescence Characteristic of Quercetin–Nanoporous Anodic Aluminum Oxide Composites

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Nanoporous Anodic Aluminum Oxide (AAO), its average diameters of porous were 50 nm, was obtained by two-step electrochemical anodization aluminum process. Highly ordered luminescence arrays formed by filling AAO nanopores with quercetin molecules has been studied by fluorescent spectroscopy in this work. AAO showed stronger adsorption capability of quercetin than Al^{3+}-quercetin complex. The mechanism may be physical and chemical adsorption all together. Meanwhile, red shift of the maximum fluorescence peak of quercetin in AAO was observed. The molecular assemblies in the nanopore array are highly ordered and the fluorescence polarization dependence indicates a preferred molecular orientation along the pore axis. This maybe explains the mechanism of molecular luminescence depending on its environment.

KEY WORDS: Nanoporous Anodic aluminum oxide; quercetin; fluorescence characteristic.

INTRODUCTION

Since the surprising special performances of nanometer materials were found, there were many nanometer material fabricated by scientists and engineers and their properties in mechanics, electromagnetism, optics and other physical and chemical characters were investigated in detail [1,2]. Nanoporous Anodic Aluminum Oxide (AAO) was used broadly to make else nanometer materials as template. The size of nanohole can be controlled by adjusting the height of voltage in types of anodizing solutions. In the past decade, photochemical properties of dye adsorbed in porous silicon, sol-gel, and porous alumina matrices [3–5] have been studied. Recently, there has been a growing interest in the study of organic-inorganic nanocomposites for fluorescence properties. New materials in nonlinear optics, solid organic lasers, and optical data storage [5–8] always attract people's attention. There are two theses on nano-particles as fluorescent biological labels for ultra sensitive unconventional fluorescent analytical method [9,10]. Obviously, it is an important field in the study of molecular luminescence and adsorption on nanometer materials. The research results will provide us with some referenced value for expanding fluorescence molecules' usage, obtaining more luminescence materials and exploring new fluorescence probe basic on unconventional analysis method.

There are many kinds of fluorescence molecules in nature and synthetic substance. Quercetin and its complexes are applied widely in many fields as dyes, medicament for remedying tumors, sensitive fluorescent probes, and analytical reagents, et al. Therefore, we selected quercetin as a fluorescent adsorbate in AAO in this work for studying fluorescence characteristic of organic-inorganic nanophotonic composites. We measured excitation and emission spectra and discussed primarily the mechanism of photoluminescence. Although there are a few reports [5,11,12] about composite structures comprising organic fluorescence molecules incorporated in inorganic nanoporous material, quercetin including highly reactive hydroxyl groups was firstly used. This experiment proved for the first time that the highly reactive hydroxyl groups of quercetin could enforce the capacity

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of adsorption and chemical adsorption could exist in organic-inorganic nanocomposites. Especially, a blue shift of quercetin-Al^{3+} complex (1:1) in solution and a red shift of the maximum fluorescence peak of quercetin in AAO were observed. This phenomenon is nice to comprehend thoroughly photoluminescence characteristics and mechanism of organic-inorganic nanocomposites.

EXPERIMENTAL

Apparatus

A Shimadzu RF-540 spectrofluorimeter (Kyoto, Japan), equipped with a 150 W xenon lamp, a 1×1 cm cell, a solid sample clip and a function recorder, was employed to scan the fluorescence spectra and to measure the fluorescence intensities. The slit-width for both the excitation and emission monochromators was set at 5 nm.

Reagents

Solution of quercetin (0.01 M) was prepared by dissolving commercial sample (Quercetin, Shanghai Biochemistry Reagent Limited Company, China) in C_2H_5OH. Quercetin-Al^{3+} complex (1:1) solution was 0.01 M C_2H_5OH. All of the reagents used in this experiment were analytical pure grade and were used without further purification.

Fabrication of AAO

We have fabricated AAO template by the follow process according to the two-step electrochemical anodization [13]. Firstly, high purity (99.99%) aluminum foils were annealed at 600°C for 6 hr. Subsequently, the sample was ultrasonically degreased in acetone, and cleaned in 5% NaOH at 15°C for 20 min, and a smooth surface could be obtained. Then, the aluminum was electropolished in a 25:75 volume mixture of $HClO_4$ and C_2H_5OH. The polished Al samples were anodized at 40 V dc in 2.7 wt% (0.3 M) $H_2C_2O_4$ at 10°C, which contained the following 4 steps: (1) anodized a polished Al sheet for 30 min; (2) dissolved away the oxide film in a mixed solution of 0.2 M H_2CrO_4 and 0.4 M H_3PO_4 at 60°C for 5 min; (3) rinsed the Al sheet with deionized water, and then anodized it for 1–3 h; (4) removed oxide film, and anodized the Al sheet for 1 h again. Consequently, the Al_2O_3 template with highly ordered pores was formed. The AAO with different size range from several tens to hundreds was achieved by adjusting the height of voltage in types of anodizing solution such as sulfuric, oxalic, and phosphoric acid.

After nanopore array formation by anodization, the AAO film was peeled off from Al substrate using a saturated $HgCl_2$ solution about 30 min. Then the stand alone transparent AAO membrane with the nanopores was obtained and adhered to a microscopic glass slide as substrate. Remnant $HgCl_2$ in nanopores of AAO membrane can interfere with the adsorption capability of quercetin on AAO and quench the fluorescence of quercetin. So, the AAO membrane should be washed by distilled water and ethanol in turn repeatedly and thoroughly in order to remove $HgCl_2$. Then dried in atmosphere and the AAO membrane was ready. The nanoporous structure and appearance had been characterized by XRD and SEAM at *Key Laboratory for Magnetism and Magnetic Materials of MOE of Lanzhou Universit*y. The average diameters of porous anodic alumina used in the present work were 50 nm and the thickness of the membrane was about 20 μm.

Adsorption and Luminescence of Quercetin in AAO

The AAO dealt with above methods were soaked in quercetin solution. For more than 3 hr later, they were took out, flushed slowly by C_2H_5OH for getting rid of adhesive solution on surface and dried in the air at room temperature for more than half an hour. Then we scanned their fluorescence spectra by spectrofluorimeter.

RESULTS AND DISCUSSION

Adsorption Capacity

We tested the interactions of quercetin with Al^{3+} by the intensity varying of fluorescence in solution. Quercetin and Al^{3+} can produce a new complex and Al^{3+}can enhance the fluorescence intensity of quercetin in solution (Fig. 1b and c). The composite quercetin-AAO surface becomes yellow stained from the originally transparent AAO membrane; whereas the chroma by human eyes is weak when the AAO membrane was soaked in Al^{3+}-quercetin complex (1:1) solution according to above methods. Moreover, the excitation spectra of two quercetin-AAO composite samples were scanned and the spectra showed the same results (Fig. 2b and c). The difference of relativity intensities means that adsorption capacity is different. In other words, the adsorption capacity of AAO varies with different molecules. The result provides us with information on adsorption mechanism.

Highly reactive hydroxyl groups of quercetin can bond onto AAO surface and the molecule is easily

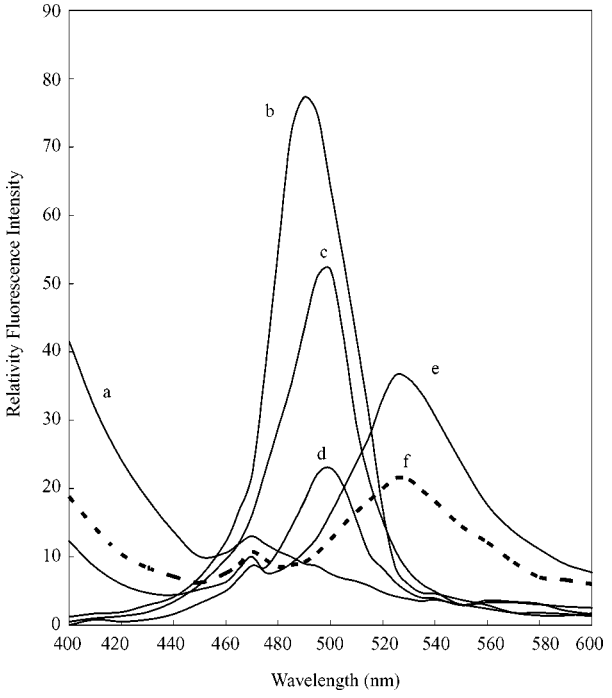

Fig. 1. Emission spectra: (a) blank 50 nm AAO, excitation wavelength (Ex) at 350 nm. (b) quercetin-Al^{3+} complex (1:1) solution (1.0×10^{-4}M, C_2H_5OH), maximum Em 490 nm, Ex 450 nm; (c) quercetin solution (1.0×10^{-4} M, C_2H_5OH), maximum Em 500 nm, Ex 450 nm; (d) quercetin-Al^{3+}complex (1:1) on filter paper, maximum Em 500 nm, Ex 350 nm; (e) quercetin in 50 nm AAO, maximum Em 525 nm, Ex 350 nm; (f) quercetin on 50 nm AAO (flushed repeatedly), maximum Em 525 nm, Ex 350 nm.

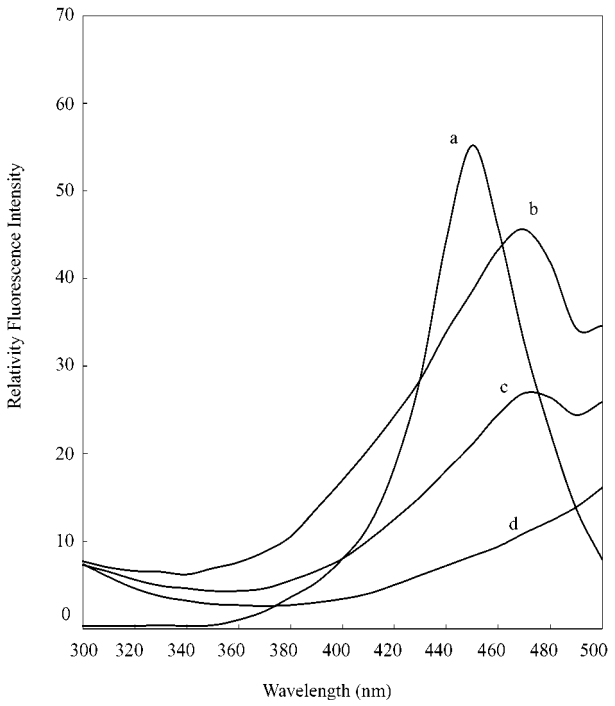

Fig. 2. Excitation spectra: (a) quercetin in ethanol solution (1.0×10^{-4} M), emission wavelength (Em) at 500 nm; (b) quercetin-AAO composites (AAO membrane soaked in quercetin-Al^{3+} complex solution), Em at 550 nm; (c) quercetin-AAO composites (AAO membrane soaked in quercetin solution), Em at 550 nm; (d) blank AAO membrane, Em at 550 nm.

adsorbed on AAO [14,15]. The quercetin molecules are tightly fitted on AAO membrane and difficultly washed. Meanwhile, when quercetin-Al^{3+} complex solution was used, part hydroxyl groups of quercetin interact with Al^{3+} and activity of quercetin decreases. Therefore, adsorption capacity of quercetin-Al^{3+} complex on AAO is weaker than that of quercetin on AAO. Based on the performance, we summarize that the mechanism is a cooperative effect of physical and chemical adsorption.

Excitation Spectra of Quercetin in AAO Samples

Quercetin in solution has fluorescence around 500 nm when excited at 450 nm (Fig. 2a and Fig. 1c). There are probably slight changes (1–3 nm) on peaks' positions in different testing surroundings and conditions. When we scanned the excitation and emission spectra of quercetin in AAO sample, we did not use the corresponding maximum emission and excitation wavelength in order to avoid or decrease interference from specular and diffuse light from solid. One of the principles of molecule fluorescence is that maximum fluorescence peak's position is invariable with the variably excitation wavelength and vice versa [16]. The spectra of quercetin were hereby confirmable.

The curve Fig. 2d shows excitation spectrum of the AAO membrane (emission wavelength at 550 nm). AAO matrix is almost transparent in the range 300–500 nm and the relativity intensity near 500 nm is stronger because of light dispersion on solid sample. The curve Fig. 2b shows excitation spectrum of quercetin-AAO sample and the peak at about 470 nm contributes to quercetin deducting background intensity from the AAO membrane. A red shift is observed comparing with the curve Fig. 1a which shows excitation spectrum of quercetin in ethanol solution (maximum peak at 450 nm, 10^{-4} M). It is the same tendency with red shift of emission spectrum in the following.

As it follows from the exciton theory [17], the dipole-dipole interaction between dye molecules can lead to *H* or *J* dimer and aggregate formation, which results in a blue or red band shift (or band splitting for oblique *J* aggregates) with respect to the monomer absorption. Indeed, excitation band is an important part of absorbance

spectra for fluorescence molecules because excitation state of molecules comes from absorbing photons. Therefore, the excitation spectrum of the quercetin in solution exhibits the typical features of oblique *J* aggregates and quercetin molecules in AAO membrane are mostly monomer-like. This ordered monomer array attributes to highly ordered nanopores and spatial confinement. More detailed explanations will be given in the following part.

Emission Spectra of Quercetin in AAO Samples

Emission spectra of quercetin in AAO membrane and reference samples and their maximum wavelengths are displayed in (Fig. 1). The spectra show that red shift is prominent when the fluorescence molecules are arranged into nanometer holes of AAO. We assume the process may be the following.

The nanopore axial symmetry and ordering have likely led to the dye orientation predominantly along the nanopore axis due to the vectorial capillary forces (from the AAO surface to the pore depth) governing the filling process [5]. The dye filling of the AAO nanoscale pores provides the selected orientation of adsorbed molecules inside nanopores, making this process similar to the Langmuir-Blodgette technique. The nanometer porous structure of AAO membrane leads to a large surface area than a film of the same size but without pores. The average size of the quercetin molecule is approximately in the order of 1–2 nm, which is substantially smaller than the pore diameter (50 nm). The large surface and highly ordered structure and cooperated effect of physical and chemical adsorption allow quercetin molecules self-assemblies to form monomer-like ordered layer and help to suppress aggregation. The interaction of fluorescence molecules in solids induces a strong quenching effect due to the formation of nonemissive dimer, trimers, and aggregates [17]. The excitation and emission spectra of pure quercetin solid powder do not present a characteristic peak or band. The fluorescence peak position of molecules in the solid state usually depends on excitation wavelength due to aggregation effect and inhomogeneous broadening. The dipole-dipole interaction between the dye molecules usually induce the exciton coupling leading to splitting of the energy levels [17]. It leads to reduce the energy difference of the lowest unoccupied molecular orbits and the highest occupied molecular orbits. Therefore a red shift occurs.

The curve Fig. 1b shows the peak of quercetin-Al^{3+} complex (1:1) solution. The peak's position is about 490 nm, which is 10 nm less than that of the curve Fig. 2c. We put a piece of ordinary filter paper in this solution for one minute and took it out. After it dried, we scanned the emission spectrum of quercetin-Al^{3+} complex (1:1) on the paper and got the curve Fig. 1d. The peaks' positions of the curve Fig. 1(c) d are less than that of curve Fig. 1e. It can be concluded that the interaction of AAO and quercetin is in essence different from that of quercetin-Al^{3+} complex in the solution. Free Al^{3+} in solution can combine with quercetin molecules completely and quercetin-Al^{3+} complex (1:1) form. In these complex molecules, the electronic field of Al^{3+} decreases the energy level difference of the highest occupied molecular orbits and the lowest unoccupied molecular orbits of quercetin. Hence, a blue shift is observed. However, there is no free Al^{3+} in solid AAO membrane because of covalent bond. The interaction between quecetin and AAO does not form complex and only leads to form composite. The decisive factor is probably that the highly ordered nanoporous array changes the molecule aggregation form.

The curve Fig. 1a shows the wider emission spectrum of blank 50 nm AAO and the band at 470–400 nm owes to light scattering (excited at 380 nm). Although the band at 470–400 nm is strong, the relative intensity is faint when the nanoholes in AAO are filled with quercetin. It is estimated that an energy transfer takes place between AAO light scattering and quercetin absorbance. The fact that wider band of AAO emission spectrum overlays the absorbance spectrum of quercetin results in that scattering light of AAO are mostly absorbed by quercetin molecules and weakens the emission band intensity of AAO. Meanwhile, the enhancement of absorbance efficiency can be used as a reason to explain the stronger fluorescence efficiency.

When quercetin-AAO composite was soaked in ethanol for hours and flushed repeatedly, the band at about 530 nm of the emission spectrum of quercetin-AAO decreases or disappears and the band at 470–400 nm increase. The curve Fig. 2(f) shows emission spectra of quercetin on 50 nm AAO in the flushing process. With molecules removed in nano-porous alumina, the characteristic of emission spectra changes gradually into scattering light of AAO from fluorescence of quercetin.

CONCLUSION

Highly ordered arrays of quercetin-AAO nanocomposites exhibit unfamiliar characteristic of adsorption and excitation and emission spectra of quercetin on nanoporous AAO membrane. We had found variations in the maximum emission peak's position and adsorption capacity of quercetin on different sorption carriers. The mechanisms of adsorption capacity and variation of

fluorescence intensity and position were primarily discussed. The highly ordered array structure of dye-AAO nanocomposites could be used to explain the phenomena on a certain extent. It is a significative work for understanding thoroughly molecule luminescence, searching better sorption carrier and prolonging application of fluorescence probe in analytical science.

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