Photoluminescence spectra of human serum albumen and morin embedded in porous alumina membranes with ordered pore arrays

2003 J. Phys.: Condens. Matter 15 8271

(http://iopscience.iop.org/0953-8984/15/49/006)

INSTITUTE OF PHYSICS PUBLISHING JOURNAL OF PHYSICS: CONDENSED MATTER

J. Phys.: Condens. Matter **15** (2003) 8271–8279 PII: S0953-8984(03)65739-5

Photoluminescence spectra of human serum albumen and morin embedded in porous alumina membranes with ordered pore arrays

Run-Ping Jia[1], Yan Shen[1], Hai-Qing Luo[1], Xing-Guo Chen[1,3], Zhi-De Hu[1] and De-Sheng Xue[2]

[1] Department of Chemistry, Lanzhou University, Lanzhou 730000, People's Republic of China
[2] Key Laboratory for Magnetism and Magnetic Materials of MOE, Lanzhou University, Lanzhou 730000, People's Republic of China

E-mail: chenxg@lzu.edu.cn and jrpchem@vip.sina.com

Received 2 July 2003, in final form 22 September 2003
Published 25 November 2003
Online at stacks.iop.org/JPhysCM/15/8271

Abstract

For the first time, an organic dye, morin, and another bio-macromolecule, human serum albumen (HSA), were embedded in porous alumina (P-Al) fabricated by anodization in oxalic acid. The photoluminescent (PL) spectra of morin and morin–HSA immersed in the P-Al pores were measured and compared with those of liquid solutions. It was found that their PL positions are similar to those of dye-Al^{3+} in ethanol solution, and the PL of the embedded morin was greatly enhanced by the introduction of HSA. We have ascribed the appearance of PL bands to the formation of morin–Al complexes in the nanopores, with its inner wall being involved. A likely luminescence model was proposed to explain the observed PL enhancement due to the coexistence of morin and HSA in the nanopores, which has been proved by UV and Fourier transform infrared measurements. Moreover, the PL intensity increased with increasing pore diameter, which may be used to develop a luminescent array to predict the pore size of a P-Al layer.

1. Introduction

Nanochannel-array materials, which have uniform channels with nanoscale diameters, have stimulated considerable interest in recent years due to their utilization as hosts or template structures for magnetic, electronic and optoelectronic devices [1, 2]. Anodic porous alumina (P-Al), a typical self-ordered nanochannel material formed by anodizing Al in an appropriate acid solution, has recently attracted increasing interest as a key material for the fabrication of nanometre-scale structures [3, 4]. Most research has focused on the growth

[3] Author to whom any correspondence should be addressed.

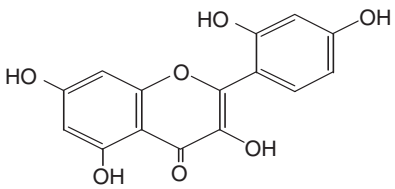

Figure 1. Structure of morin.

process and fabrication mechanism [5, 6]. In recent years, there has been growing interest in incorporating laser dyes into solid media for device applications, and some corresponding luminescent mechanisms have been discussed [7–10]. However, there have been no reports on the optical properties of bio-macromolecules, such as human serum albumen (HSA), on the P-Al membrane after dipping in dye solution.

As a very sensitive organic agent, morin (3,5,7,2′,4′-pentahydroxyflavone, whose structure was shown in figure 1) is only weakly fluorescent, while the fluorescence of the morin–Al^{3+} complex is very strong [11]. Based on this, a sensitive method for the determination of aluminium was established, with a detection limit in the parts-per-billion range [12]. In addition, this dye also has been used as a sensitive probe for other metal ions [13, 14] and some bio-macromolecules, such as protein [15] and nucleic acid [16] in the liquid phase. Up to now, the optical properties of morin and morin–HSA embedded in a P-Al membrane have not been paid much attention, so we investigated their photoluminescent (PL) behaviours in this work. The motivation of this study is to draw attention to the development of a high density luminescent array based on P-Al. Moreover, it may be possible to develop a novel approach for the detection of protein using the P-Al matrix. This study may lead to a better understanding of the energetics, potential distribution and carrier dynamics in these fascinating nanostructured materials. In addition, mechanisms for the characterization behaviours of the PL band are also discussed.

2. Experimental details

2.1. P-Al preparation

High purity (99.999%) aluminium (dimensions 30 mm × 10 mm, thickness 1 mm) was used as the starting material. Before anodization the aluminium was annealed at 600 °C for 6 h and then degreased ultrasonically with acetone and cleaned in 5% NaOH at 15 °C for 20 min. After that, the aluminium was electropolished in a 25:75 by volume mixture of $HClO_4$ and C_2H_5OH. P-Al membranes were prepared in a two-step anodizing process [17]. The polished alumina foil was anodized at a constant voltage of 80 V in a 0.5 mol l^{-1} phosphoric acid (H_3PO_4) electrolyte bath at room temperature for 3 h, to obtain P-Al film with an average pore size of 100 nm. Then the specimens were immersed in a mixture of 6 wt% H_3PO_4 and 1.8 wt% H_2CrO_4 at 60 °C for 5 min to remove the alumina layers. The alumina foil was then anodized again for 1 h under the same anodization conditions as the first step. Different thicknesses of P-Al film, ranging from several tens to hundreds of nanometres were achieved by adjusting the voltage in various types of anodizing solution such as sulfuric, oxalic and phosphoric acid.

The morphology of the as-prepared P-Al membranes was observed using a JSM-5600LV scanning electron microscope (SEM). The average diameters of the P-Al used in the present work were 50, 75 and 100 nm.

2.2. Absorption of dye and human serum albumen

Morin was purchased from a Beijing chemical plant. The stock solution of morin (1.0×10^{-2} mol l^{-1}) was prepared by dissolving it in 95% ethanol. HSA (from Sigma, St Louis, MO) was directly dissolved in deionized water to prepare a stock solution of 1000 μg ml^{-1}, which was kept at 0–4 °C. Working solutions with lower concentrations were freshly prepared by appropriate dilution of the stock solution with water prior to use. A pH 4.5 acetic acid–sodium acetate buffer solution was used to control the acidity of the aqueous system.

The procedure started by placing 1.0 ml of pH 4.5 buffer in a 10 ml flask, adding an appropriate volume of morin and mixing. Then an appropriate volume of HSA was added and mixed, and similarly an appropriate volume of ethanol. This was made up to volume with water and mixed thoroughly to prepare 50% ethanol soaking solutions containing morin and HSA in various concentrations. The P-Al films with a remaining aluminium layer were dipped in above-prepared soaking solutions for different soaking times. Then they were removed, flushed fully with 50% ethanol and dried.

2.3. Spectra measurements

Photoluminescence spectra of all the samples were measured at room temperature by a Hitachi M-850 fluorescence spectrophotometer with a Xe lamp as the excitation light source. The absorption spectra were measured with a Shimadzu UV-240 ultraviolet–visible spectrophotometer. The Fourier transform infrared (FTIR) spectra of all the samples were measured by a Nicolet Nexus 670 FT-IR spectrometer equipped with a germanium attenuated total reflection (ATR) accessory, a DTGS KBr detector and a KBr beam splitter.

3. Results and discussion

3.1. Characterization of the porous alumina membrane

A scanning electron micrograph of a P-Al template is shown in figure 2(A), from which it was found that the nanopores are uniform and highly ordered with diameters of about 100 nm. The pore distribution graph further proved the pore size was about 100 nm, as shown in figure 2(B). It indicates that the pore sizes are in the range 0.090–0.110 μm and the dominant pore sizes range from about 0.095–0.105 μm.

3.2. Spectra analysis

The emission spectra of morin, morin–Al^{3+}, morin–HSA and morin–Al^{3+}–HSA ethanol solutions were investigated and the results are presented in figure 3(A). As expected, morin in 50% ethanol solution is nearly non-fluorescent (curve (a)), while the morin–Al^{3+} complex is highly fluorescent (curve (c)). Additionally, the introduction of HSA hardly changed the PL position and intensity of morin in ethanol solution (shown in curve (b)). Moreover, the morin–Al^{3+}–HSA complex (curve (d)) showed an obvious PL band near 500 nm, the position being similar to that of morin–Al^{3+}, and its PL intensity was enhanced greatly relative to that of morin–HSA. As we know, the P-Al film itself has a blue PL band originating from singly ionized oxygen vacancies (F^+ centre) in P-Al membranes [18] but it does not present the PL band under the measured conditions. However, when dye or dye–HSA molecules were confined in nanometre-sized holes, an evident PL band near 500 nm appeared, and the intensity of the embedded dye was much weaker than that of embedded dye–HSA, as shown in figure 3(B). Furthermore, their PL peak positions shifted to a shorter wavelength relative to that

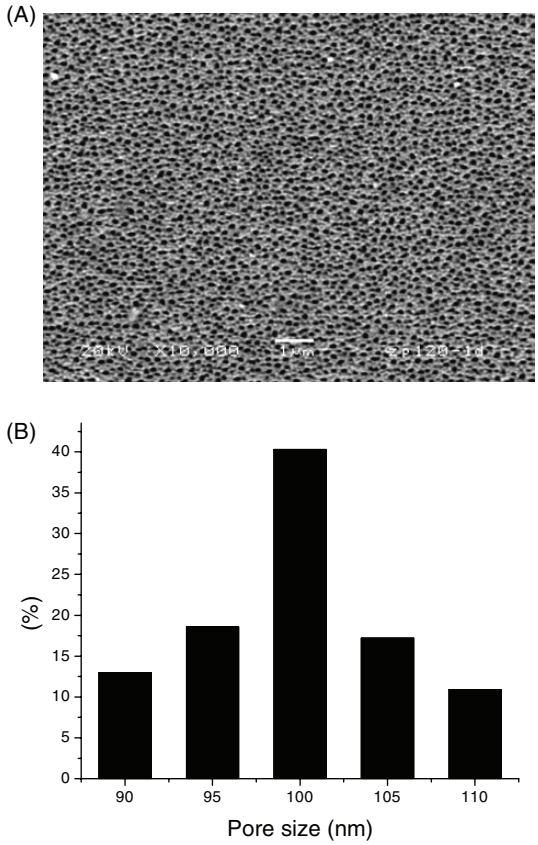

Figure 2. (A) SEM image of porous anodic alumina film. (B) Pore distribution graph of the P-Al film with 100 nm pore size.

of the liquid morin–Al^{3+} complex. Considering the nano-effect of P-Al film and comparing with figure 3(A), it was considered that the peak shift is attributable to the aggregates of dye or dye–HSA buried in the nanometre-scale pores, because dimers and multimers can form due to strong interactions between molecules in the liquid phase. As a result, the gap between the excited states of the molecules and phonons is increased, so a red shift in the PL is observed [19]. However, when the molecules are buried in a nanometre-sized hole, the interaction between the molecules is reduced, thus the PL band moves towards a shorter wavelength.

The immersing sequence of the P-Al layer has some effect on the PL intensity. No P-Al emission signal was detected when the film was dipped in a solution containing protein only. An evident PL band near 500 nm appeared by further dipping it in dye solution for some time. In addition, immersing the P-Al film in morin solution first, a weak PL was found; then immersing it in HSA solution, the PL was enhanced, but its intensity was weaker than immersing it directly in a mixed solution of morin and HSA. Based on the above phenomena, we inferred that the PL appearance and enhancement were due to morin and morin–HSA embedded in the nanopores. A subsequent cleaning treatment of the P-Al was undertaken. Thoroughly rinsing them either with deionized water or 50% ethanol, or even acetone, the PL intensity was hardly weakened, especially the latter, with morin–HSA. Only when ultrasonically treated for 2 h, did the PL intensity show any obvious weakening. Even by extending the ultrasonic cleaning time to

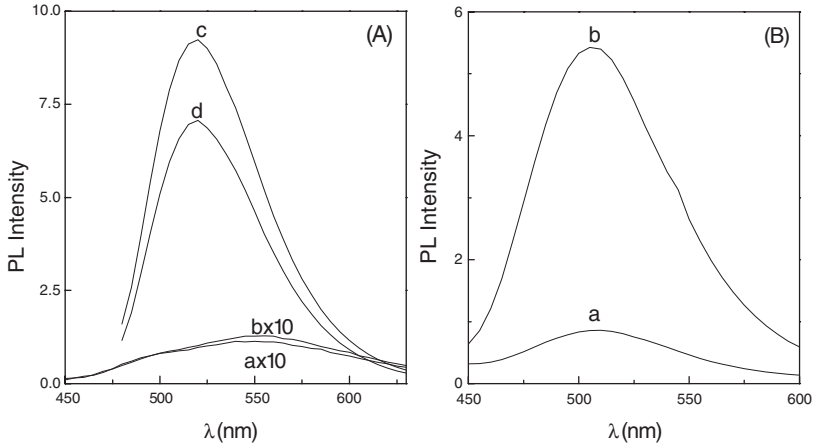

Figure 3. (A) Emission spectra of (a) morin, (b) morin–HSA, (c) morin–Al^{3+} (excited at 470 nm) and (d) morin–Al^{3+}–HSA (excited at 470 nm) in 50% ethanol solutions. (B) Emission spectra of P-Al after absorption in (a) dye and (b) dye–HSA 50% ethanol solutions. Concentrations: morin 1×10^{-3} mol l^{-1}, HSA 200 μg ml^{-1} and Al^{3+} 1.38×10^{-4} mol l^{-1} with $\lambda_{ex} = 420$ nm.

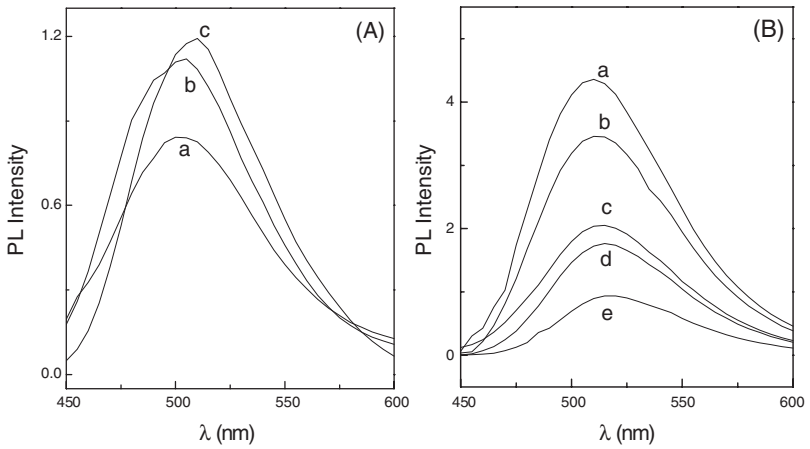

Figure 4. (A) PL spectra of P-Al after absorption in morin and HSA ethanol solution with pore diameters of (a) 50 nm (b) 75 nm and (c) 100 nm. (B) PL spectra of P-Al after absorption in morin and HSA ethanol solution for (a) 0.5 h, (b) 1 h, (c) 2 h, (d) 5 h and (e) 7.5 h. Concentrations: morin 2×10^{-4} mol l^{-1} and HSA 200 μg ml^{-1} with $\lambda_{ex} = 420$ nm.

over 5 h, the PL bands did not completely disappear. This suggested that the absorption of morin and HSA in the P-Al pores depended not on a single physical absorption, but mainly on a chemical reaction with the inner wall of the pores involved. Hence, in all subsequent work, the P-Al film was either immersed in a solution containing dye and HSA or not.

To reveal the effect of the pore size on the PL position and intensity, we investigated the PL of morin–HSA with different pore sizes and the results are summarized in figure 4(A). Obviously, the PL intensity increased with increasing pore size. Additionally, the PL band moved towards longer wavelength, which is attributed to the aggregation of molecules in a nanometre-sized hole. The molecules are more easily packed in larger hole, so dimers and multimers are more easily formed than in a smaller hole. In other words, the PL of molecules

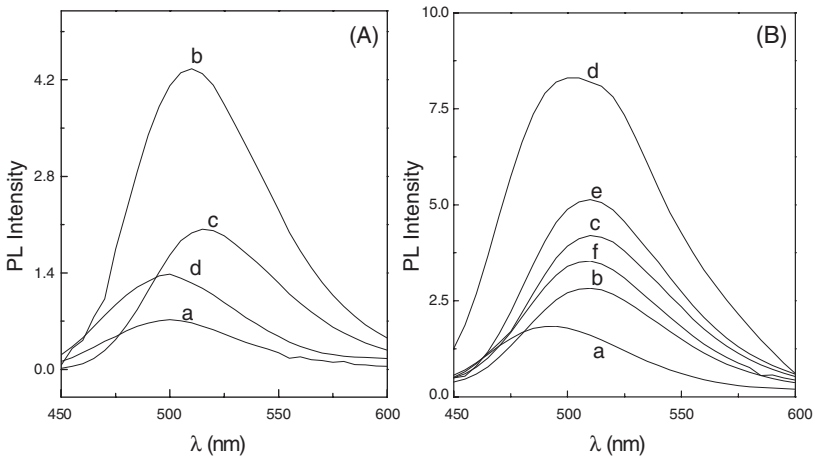

Figure 5. (A) PL spectra of P-Al after absorption in dye and HSA (200 μg ml^{-1}) ethanol (50%) solutions with dye concentrations of (a) 5×10^{-5} mol l^{-1}, (b) 2×10^{-4} mol l^{-1}, (c) 1×10^{-3} mol l^{-1} and (d) 4×10^{-3} mol l^{-1}. (B) PL spectra of P-Al after absorption in dye (2×10^{-4} mol l^{-1}) and HSA ethanol (50%) solutions with HSA concentrations of (a) 0 μg ml^{-1}, (b) 40 μg ml^{-1}, (c) 120 μg ml^{-1}, (d) 200 μg ml^{-1}, (e) 300 μg ml^{-1} and (f) 400 μg ml^{-1}, and $\lambda_{ex} = 420$ nm.

in a nanometre-sized hole resembles that of monomers, but in a larger hole, the spectrum is closer to that of molecules in a high-concentration solution or film, thus the PL shifts to longer wavelength. So a pore size of 100 nm was used in subsequent experiments. Figure 4(B) showed the influence of soaking time on the PL intensity and position. Clearly, the PL intensity showed a distinct decrease with longer absorption time. At the same time, an obvious red shift was observed, so 30 min of soaking time was chosen in subsequent work.

Figure 5 shows the effect of dye or HSA concentration in the soaking solution on the PL intensity. As shown in figure 5(A), the intensity of the PL band increased with increasing dye concentration at first, reached a maximum at a dye concentration of 2×10^{-4} mol l^{-1}, and then decreased. The maximum PL enhancement was located at 120 μg ml^{-1} HSA, as shown in figure 5(B). This also shows that there is a positive correlation between the PL intensity and HSA content in a certain concentration range. It is a pity, however, that it is not a linear relationship.

Figure 6 shows the absorption spectra of morin and HSA in solution and in P-Al films. As shown in figure 6(B), P-Al film by itself showed no characteristic absorption at 400 nm. However, when dye or dye–HSA molecules were absorbed on the inner wall of cylindrical pores, the absorption band around 400 nm appeared. Similar results, as shown in figure 6(A), were observed from the liquid samples of dye and dye–HSA. This suggested that the PL band near 500 nm does not originate from the P-Al but from dye or dye–HSA impregnated in the nanopores.

The FTIR measurements further proved the presence of dye and HSA in the nanopores, as shown in figure 7. It was clear that the P-Al film by itself has no characteristic absorption in the region 1400–1800 cm^{-1}, as shown in curve (a). The presence of morin in the nanopores was confirmed by the characteristic absorption near 1730 cm^{-1}, which originates from the C=O group in the morin molecule, as illustrated in curves (b) and (d). The inclusion of HSA was characterized by the strong C=O stretch band of the amide group (amide I) near 1650 cm^{-1} and the band near 1540 cm^{-1} that involves both C–N stretch and C–N–H in-plane bending in the stretch-bend mode (amide II), as shown in curves (c) and (d).

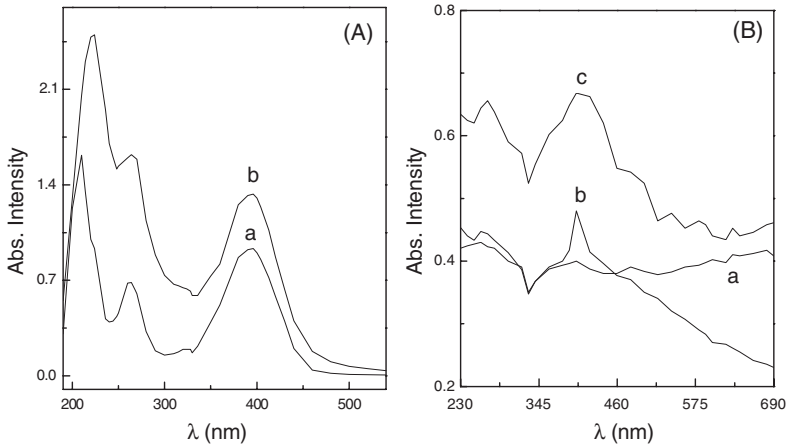

Figure 6. (A) UV absorption spectra of (a) morin and (b) morin–HSA in 50% ethanol solutions. (B) UV absorption spectra of P-Al (a) as prepared, and (b) and (c) after absorption in morin and morin–HSA 50% ethanol solution, respectively. Concentrations: morin 2×10^{-4} mol l^{-1} and HSA 120 μg ml^{-1}.

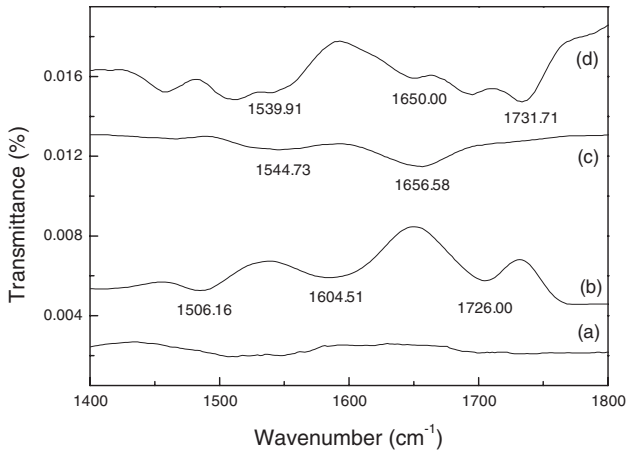

Figure 7. FTIR spectra of P-Al film (a) as prepared, and (b), (c) and (d) after absorption in dye, HSA and dye–HSA 50% ethanol solution, respectively. Concentrations: morin 1×10^{-3} mol l^{-1} and HSA 200 μg ml^{-1}.

The P-Al film obtained by anodization primarily consisted of disordered Al_2O_3, in which there are large numbers of nanometre-sized cylindrical pores. So it may show strong surface effects [20] due to its colossal specific surface area. That is, there must be numerous aluminium atoms on the inside surface of the P-Al pores, which are characterized by their high reactivity and instability due to being under-coordinated and having high surface energy. Therefore, when dye molecules are introduced into the cylindrical P-Al pores, they may react with the remaining aluminium on the inside surface of the holes to form the morin–Al complex. However, being confined in a nanometre-sized hole, the binding of aluminium to dye might be restricted to certain directions only and may not form the fully-coordinated complex as freely as those in solutions, so its PL intensity is much weaker than that of morin–Al^{3+} in the liquid phase.

The spatial configuration of protein is described as being composed of three homologous domains linked together by peptide chains. Almost all the hydrophobic amine acid residues are placed between the 'helices' and inside the trough, whereas the great majority of polar residues are on the outer wall of the structure. So the cylindrical domain has a hydrophobic interior and a polar exterior [21]. In a pH 4.5 buffer solution, morin binds to protonated amine groups of amino acid residues in the polypeptide chain to form the dye-protein complex, which shows an increased absorption relative to that of dye (see figure 6(A)). As for dye–HSA impregnated in a nanohole, a likely luminescence model is proposed to explain the PL enhancement. In this case, we infer that the remaining aluminium on the inside surface binds to the dye molecules at first and, subsequently, binds in an orderly fashion to protein molecules assumed to be located in the middle of the cylindrical cavity of the pores. Thus, an orderly, stable and spatial system consisting of HSA, morin and the inner wall of pores involved is formed. Its binding order is inner wall–morin–HSA–morin–inner wall and almost all dye-Al complexes are ordered around the polar exterior of the protein molecule. This orderly arrayed system can show a much higher luminescent efficiency relative to the disordered dye-Al complex in a nanometre-sized hole, thus a PL enhancement, with the PL position unchanged, can be observed. No HSA molecule, no orderly spatial system. This may be why the PL intensity of embedded dye can be enhanced by the introduction of HSA.

4. Conclusion

In this study, the PL spectra of morin and HSA absorbed in the pores of P-Al were investigated. It was found that their PL bands are similar to those of liquid dye-Al^{3+} complex, which may be attributed to the interaction of morin with the remaining aluminium in the nanopores. Moreover, the PL intensity of the embedded dye was enhanced greatly by the introduction of HSA. We inferred that the PL enhancement may be due to the coexistence of dye and HSA in the pores, and this has been proved by UV and FTIR measurements. Based on this, a likely luminescence model was proposed. We also found that the PL intensity increased with increasing pore size. Although more work is still needed to understand the underlying mechanisms, we believe that this new type of material is very promising for optoelectronic and biosensor applications.

Acknowledgments

This work was supported by the National Natural Scientific Foundation of China (No 20275014) and the Visiting Scholar Foundation of Key Laboratory in Lanzhou University.

References

[1] Ono S J, Ichinose H and Masuko N 1992 *J. Electrochem. Soc.* **130** L80
[2] Masuda H, Yamada H, Satoh M and Asoh H 1997 *Appl. Phys. Lett.* **71** 2770
[3] Masuda H and Fukuda K 1995 *Science* **268** 1466
[4] Hideki M, Haruki Y, Masahiro S and Hidetaka A 1997 *Appl. Phys. Lett.* **71** 2770
[5] Alwitt R S and Dyer C K 1982 *J. Electrochem. Soc.* **22** 711
[6] Chen W and Tang H G 1995 *Appl. Phys. Lett.* **67** 317
[7] Xu C X, Xue Q H, Zhong Y, Cui Y P, Ba L, Zhao B and Gu N 2002 *Nanotechnology* **13** 47
[8] Wu J H, Wu X L, Tang N, Mei Y E and Bao X M 2001 *Appl. Phys.* A **72** 735
[9] Shi Y L, Wang Z and Li H L 2001 *Appl. Phys.* A **71** 1
[10] White C E and Lowe C S 1940 *Ind. Eng. Chem. Anal.* ED **12** 229
[11] Saarl L A and Seltz W R 1983 *Anal. Chem.* **55** 667
[12] Fritz W III 1961 *Anal. Chem.* **33** 1360

[13] Xia C B, Shi H W, He X Z and Huang N D 2001 *Fenxi Ceshi Xuebao* **20** 54
[14] Engelborghs Y 2001 *Spectrochim. Acta.* A **57** 2255
[15] Zhao F L, Wang Y T, Liu F F and Li K A 2001 *Acta Sci. Nat. Univ. Pekin.* **37** 741
[16] Wang Y T, Zhao F L, Liu F F and Li K A 1999 *Fresenius J. Anal. Chem.* **364** 560
[17] Masuda H and Satoh M 1996 *Japan. J. Appl. Phys.* **35** L126
[18] Li P, Li Q S, Ma Y R and Fang R C 1996 *J. Appl. Phys.* **80** 490
[19] Bomchil G, Haramoto A and Sakata T 1998 *Chem. Phys. Lett.* **148** 215
[20] Cavicchi R E and Silsbee R H 1984 *Phys. Rev. Lett.* **52** 1453
[21] Ulrich K H 1981 *Pharmacol. Rev.* **33** 17