

One- and Two-photon Excited Fluorescence of Zinc(II), Cadmium(II) Complexes Containing Phenothiazine Ligand

Zhao Ming XUE^{1*}, Yu Peng TIAN^{1,2}, Dong WANG², Min Hua JIANG²

¹Department of Chemistry, Anhui University, Hefei 230039

²State Key Laboratory of Crystal Materials, Shandong University, Jinan 250100

Abstract: A new ligand, 10-ethylphenothiazinyl-3-yl-methylene thiosemicarbazone (HL) and its complexes ML_2 ($M=Zn^{2+}$, Cd^{2+}), which exhibit intensive two-photon excited (TPE) fluorescence at 800 nm laser pulses in femtosecond regime, were synthesized and characterized. The measured power dependence of the fluorescence signals provided direct evidence for TPE. All of them exhibited a large two-photon absorptive cross section and, more importantly from the application point of view, high photochemical/photothermal stability.

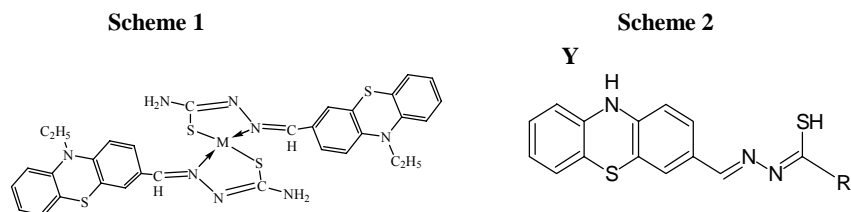
Keywords: OPE, TPE, complex, phenothiazine.

In fluorescence imaging, two-photon excitation (TPE) has developed as an important alternative to traditional one-photon excitation (OPE) in fluorescence microscopy and spectroscopy^{1,2}. The intrinsic advantages of the two-photon excitation include reduced background fluorescence from fluorophores outside the focal volume, decreased photobleaching, inherent optical sectioning capability, and lower photodamage of sensitive biological sample³. But, all the reported materials, which exhibit a strong TPE, are only organic chromophore⁴. To our knowledge, no metal complex that exhibits an efficient TPE is reported till now. In this paper, we present the two-photon properties chromophore: 10-ethylphenothiazinyl-3-yl-methylene thiosemicarbazone (HL), and its complexes (ZnL_2 , CdL_2) that exhibit intensive two-photon excited fluorescence. They were prepared and fully characterized by MS, ¹HNMR, IR, and elemental analysis. The structures of HL, ZnL_2 , CdL_2 (**Scheme 1**) are shown bellow.

The electronic absorption spectra of HL and ML_2 shown in **Figure 1**, were measured in DMF solution ($\sim 10^{-5}$ mol/L). All the compounds (HL and ML_2) exhibit essentially the same absorption profile: an intense ($\lg \epsilon = 4\sim 5$), three absorption peaks and no linear absorption in the spectral range from ~ 500 to 800 nm.

Compounds HL and ML_2 emit a green color when irradiated (one-photon processes) by UV in DMF solution (shown in **Figure 1**). All the complexes show the similar features: exciting λ_{max}^{ex} (395 nm), emission band λ_{max}^{em} (505 or 510 nm) resembling to that of HL λ_{max}^{em} (520nm), and a large stokes shift (~ 120 nm). At the same time, they show

*E-mail: zmxue@ahu.edu.cn



λ_{max}^{em} changes similar to λ_{max} of absorption spectra in **Figure 1** as $\lambda_{max}^{em}(\text{HL}) > \lambda_{max}^{em}(\text{CdL}_2) > \lambda_{max}^{em}(\text{ZnL}_2)$. There is a significant difference between the fluorescence emission spectra of the HL solution and ZnL_2 or CdL_2 . The ZnL_2 (CdL_2) solution emission spectra shows an asymmetric peak at 505 nm (510 nm) with low-energy tail only extending to 650 nm. But the HL solution fluorescence emission spectra shows a maximum peak at 520 nm, an additional feature at ~ 550 nm and a broad band with low-energy tail extending to 720 nm. In series HL and ML_2 , they show the order in quantum yield (ϕ_f), intensity (I), as $\text{CdL}_2(\phi_f=0.31, I=852) > \text{ZnL}_2(\phi_f=0.28, I=481) > \text{HL}(\phi_f=0.058, I=38)$. The emission spectra exhibit the similar features, revealing that $\pi\pi^*$ state of moiety Y (**Scheme 2**) in the ligand HL and its complexes ML_2 is believable to be responsible for the green luminescence.

The fluorescence lifetimes of HL, ZnL_2 and CdL_2 in DMF dilute solution excited at 395 nm were measured. The decay curves could be well fit by a single-exponential dependence with lifetime value of $\tau = 6.8, 6.7, 6.7$ ns for HL, ZnL_2 , and CdL_2 , respectively.

HL, ZnL_2 and CdL_2 excited at 800 nm in DMF solution ($\sim 1.0 \times 10^{-2}$ mol/L) with a titanium:sapphire mode-locked laser system (800 nm excitation, 76 MHz repetition rate, < 200 fs pulse width, Coherent Mira900-D) yielded the fluorescence spectra displayed in **Figure 2**. The characteristic Y bands had peaks at 520 nm for HL, 505 nm for ZnL_2 , and 510 nm for CdL_2 , and were similar to those observed with OPE excited at 400 nm. The results from fluorescence determination (**Figure 2**) show the order in intensity as $I(\text{HL}) > I(\text{ZnL}_2) > I(\text{CdL}_2)$. This order is reverse to that of OPE fluorescence intensity. Most of the emission wavelengths are away from the linear absorption band, *i.e.*, there is a large Stokes shift. However, besides with the different order, some differences emerged upon more detailed analysis of the wavelength region around 550 nm for them. For OPE excited at 400 nm, the fluorescence spectra of HL show a shoulder at ~ 550 nm, but the fluorescence spectra of ZnL_2 and CdL_2 show no shoulder, respectively. For TPE with 800 nm photons, however, the fluorescence spectra of ZnL_2 and CdL_2 show the features at 550 nm with a shoulder respectively, and that of HL shows no shoulders.

The power dependence of the fluorescence detected at 520 nm for HL, or 505 nm for ZnL_2 or 510 nm for CdL_2 was determined from a log-log plot of the fluorescence signal *versus* incident peak photon flux density. For photon flux densities $\leq 1.04 \times 10^{29}$

Figure 1 Absorption, normalized one-photon emission spectra in DMF solution

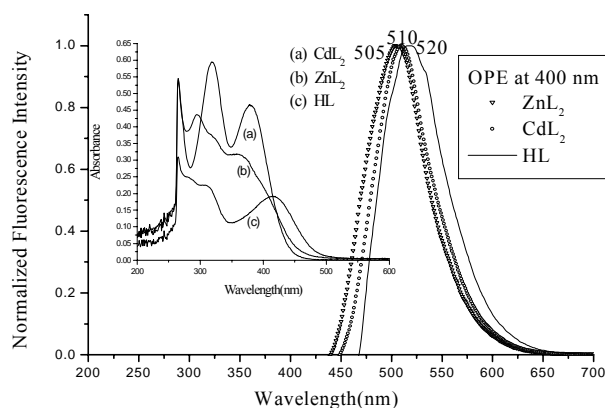
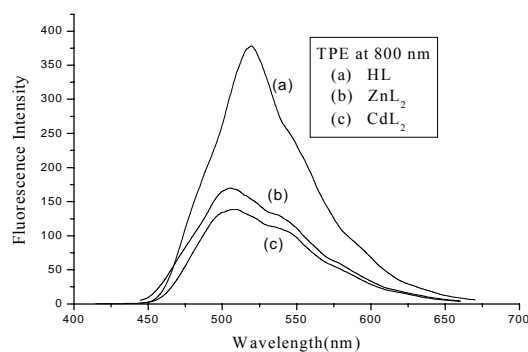


Figure 2 Two-photon emission spectra in DMF solution



for HL, $\leq 8.54 \times 10^{28}$ for ZnL_2 , and $\leq 9.86 \times 10^{28}$ photons $cm^{-2} s^{-1}$ for CdL_2 , the induced fluorescence obeyed a power-squared intensity dependence as indicated by the measured slope of 1.90, 1.90, 1.91, respectively, thereby confirming the existence of TPE. However, a decrease in the apparent power exponent was observed for larger irradiances. All measurements were carried out at the lower intensity levels at which deviations from the second-order power law were absent.

The blue emission in all three compounds observed upon 800 nm excitation was in general agreement with the fluorescence spectra excited by OPE at 400 nm. This fact, coupled to energy conservation considerations, indicates a simultaneous absorption of two 800 nm photons. Direct evidence for the TPE phenomenon was provided by the measured power-squared dependence of the induced fluorescence intensity on the incident 800 nm photon flux density.

On the basis of the facts that the emission spectra of HL, ZnL_2 and CdL_2 are nearly

the same for OPE and TPE, respectively. One may conclude that in both cases the fluorescence emission is predominantly from the same, the S_1 singlet band. As there is a considerable Stokes shift between the pump wavelength λ_0 for OPE (or $\lambda_0/2$ for TPE) and the emission wavelengths, the excited molecules must relax to the lowest excited state of S_1 singlet band before they start to emit the fluorescence. The upper state, of the molecule transition for one-photon absorption and for two-photon absorption can be different. The two-photon excitation/ absorption spectra might be drastically different from the corresponding linear excitation/ absorption spectra (in the absorbing photons energy scale) for a given solution sample. Based on these considerations, one can explain the differences between TPE and OPE fluorescence intensity orders and features.

The TPE fluorescence clearly saturated at higher flux densities, thereby establishing an upper limit for quantitative TPE microscopy of $\sim 1.04 \times 10^{29}$, $\sim 8.54 \times 10^{28}$ and $\sim 9.86 \times 10^{28}$ photons. $\text{cm}^{-2}.\text{s}^{-1}$ for HL, ZnL_2 and CdL_2 , respectively. The quenching of the excited singlet state by excited-state absorption at high power levels was the most likely cause for the observed deviation from the simple power law.

The third-order nonlinear absorption of HL and ML_2 solutions (in DMF, $\sim 10^3$ mol/L) is measured by open-aperture Z-scan technique at a 532 nm wavelength. We find that the complexes show weaker nonlinear optical properties than the legend, and the order as $\sigma_2(\text{HL}) = 0.18 \times 10^{46} > \sigma_2(\text{ZnL}_2) = 0.14 \times 10^{46} > \sigma_2(\text{CdL}_2) = 0.10 \times 10^{46}$ ($\text{cm}^4\text{s}/\text{photon}$), the order is consistent with that of intensity of TPE fluorescence excited at 800 nm.

Acknowledgments

This work was supported by the grant for the State Key Program of China (G1998061402), and the National Natural Science Foundation of China (29871001, 20071001).

References

1. K. Svoboda, W. Denk, D. Kleinfeld, D. W. Tank, *Nature*, **1997**, 385, 161.
2. J. Bewersdorf, R. Pick, S. W. Hell, *Opt. Lett.*, **1998**, 23, 655.
3. W. Denk, D. W. Piston, W. W. Webb, *In Handbook of Biological Confocal Microscopy*. J. P. Pawley, editor. Plenum Press, New York. 445.
4. Y. Zh. Shen, D. Jakubczyk, F.M. Xu, J. Swiatkiewicz, P. N. Prasad, B. A. Reinhardt, *Appl. Phys. Lett.*, **2000**, 76, 1.

Received 25 July, 2002