Chapter 5 Hydration Dynamics of BMVC in organic solvent

5.1 Introduction

Recently, carbazole derivatives have attracted lots of attentions because of its potential to be used as light-emitting materials[1,2] and anti-tumor agents.[3,4] The large variety and flexibility in molecular synthesis makes it a good candidate to be used as a molecular probe in the biological systems.[5,6,7,8] In 2003, Chang *et. al.* synthesized a novel molecule, 3,6-bis(1-methyl-4-vinylpyridium) carbazole diiodide (BMVC, the structure is shown in Figure 5.1), which can recognize the guanine-rich quadruplex structure of DNA and has potential to be used as anti-cancer drug.[9] When BMVC dissolves in tris-HCl buffer (PH=7.5), fluorescence intensity is weak and central at ~590 nm. However, as BMVC interacted with linear duplex (LD) and quadruplex DNA (Hum24), the fluorescence intensity increased more than two orders of magnitude and the emission band blue-shifted to ~560 and ~580 nm, respectively. The excited state dynamics of carbazole derivatives (3,6-DPVTCz) with similar

[1] Wu, F.-I., Shih, P.-I., Yuan, M.-C., Dixit, A. K., Shu, C.-F., Chung, Z.-M. and Diau, E. W.-G. *J. Mater. Chem.* **2005**, *15*, 4753.

[2] Morin, J.-F., Drolet, N., Tao, Y. and Leclerc, M. *Chem. Mater* **2004**, *16*, 4619.

[3] Mann, J., Baron, A., Opoku-Boahen, Y., Johansson, E., Parkinson, G., Kelland, L. R. and Nidle, S. *J. Med. Chem.* **2001**, *44*, 138.

[4] Dias, N., Jacquemard, U., Baldeyrou, B., Tardy, C., Lansiaux, A., Colson, P., Tanious, F., Wilson, W. D., Routier, S., Mérour, J.-Y. and Bailly, C. *Biochemistry* **2004**, *43*, 15169.

[5] Pal, S. K., Zhao, L. and Zewail, A. H. *Proc. Natl. Acad. Sci.* **2003**, *100*, 8113.

[6] Pal, S. K., Zhao, L., Xia, T. and Zewail, A. H. Ibid., *100*, 13746.

[7] Pal, S. K., Peon, J. and Zewail, A. H. Ibid.**2002**, *99*, 1763.

[8] Nandi, N., Bhattacharyya, K. and Bagchi, B. *Chem. Rev.* **2000**, *100*, 2103.

[9] Chang, C.-C., Wu, J.-Y. and Chang, T.-C. *J. Chin. Chem. Soc* **2003**, *50*, 185.

structures has been investigated by Wang *et. al.*[10] Both experimental and theoretical results indicated that intramolecular motion plays an important role in determining the excited state dynamics of distyryl substituted carbazole derivatives.[10]

According to Chang's recent studies,[11] the emissive state of BMVC was attributed to the intramolecular charge transfer (ICT) state, and the charge separation occurred between carbazole moiety and methyl pyridinium cation. In general, the ICT state is a nonemissive state, and the fluorescence quantum yield is affected by both solvent polarity and environment rigidity.[12,13,14]. However, those studies were limited to the steady state measurement and nanosecond (ns) time-resolved emission spectra, and the information about the excited state dynamics is still absent.

With the application of picosecond (ps) and femtosecond (fs) time-resolved fluorescence spectra, we investigated the excited state dynamics of BMVC in tris-buffer and glycerol. For BMVC in tris-buffer, we found that the fluorescence would be quenched via the rotational induced internal conversion (IC) and intersystem crossing (ISC) processes. In other words, the fluorescence intensity of BMVC in glycerol is enhanced due to the restriction of intramolecular motion. The solvation dynamics of BMVC in those two environments is also investigated. When BMVC dissolved in tris-buffer, the solvation process deviated from that in bulk water. We believed that it is due to the interaction between water and tris-HCl molecules. In glycerol, because of the relative rigid environment, the rotational motion of solvent

[10] Wang, T.-T., Chung, S.-M., Wu, F.-I., Shu, C.-F. and Diau, E. W.-G. *J. Phys. Chem. B* **2005**, *109*, 23827.
[11] Chang, C.-C., Chu, J.-F., Kuo, H.-H., Kang, C.-C., Lin, S.-H. and Chang, T.-C. *J. Lumin.* **2006**.
[12] Shin, E.-J. and Lee, S.-H. *Bull. Korean Chem. Soc* **2002**, *23*, 1309-1314.
[13] Soujanya, T., Fessenden, R. W. and Samanta, A. *J. Phys. Chem.* **1996**, *100*, 3507.
[14] Willets, K. A., Nishimura, S. Y., Schuck, P. J., Twieg, R. J. and Monerner, W. E. *Acc. Chem. Res.* **2005**, *38*, 549-556.

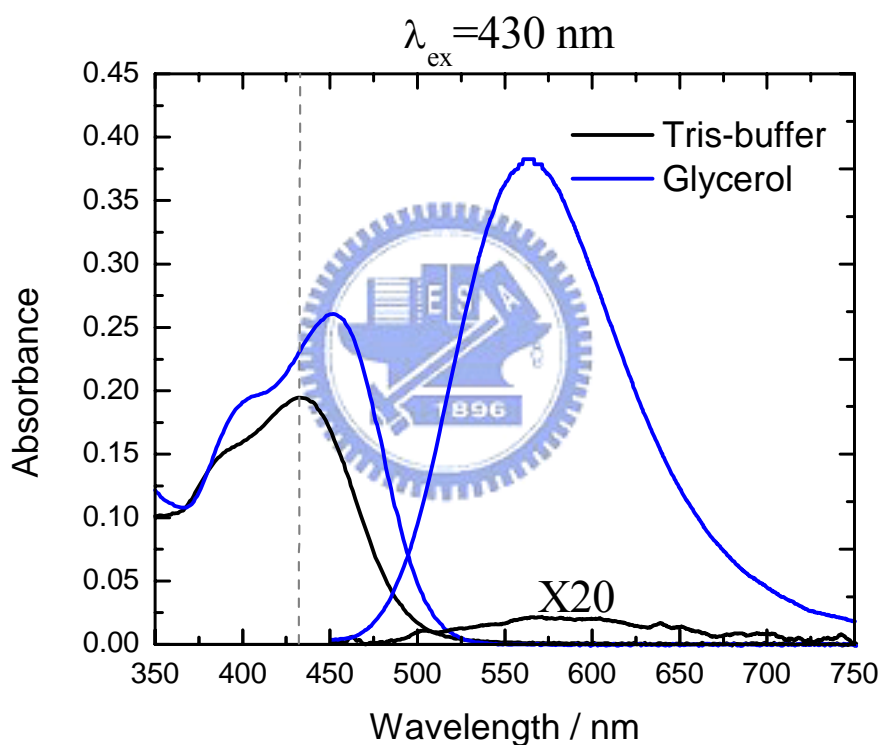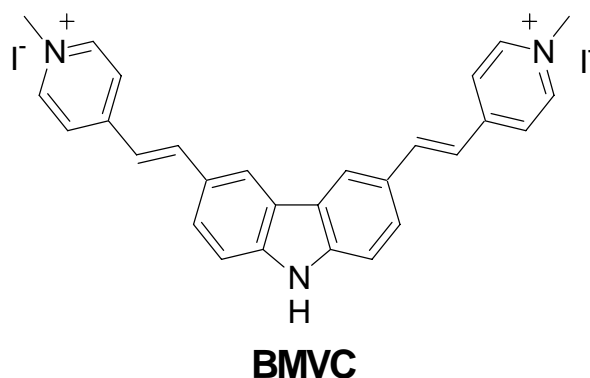molecule is restricted, and the solvation process is decelerated.

BMVC

Figure 5.1: The steady state UV and PL spectra of BMVC in tris-buffer and glycerol. The concentration of each sample is fixed at 5×10^{-6}M, and the excitation wavelength is fixed at 430 nm.

5.2 Experimental section

BMVC is synthesized through the method described elsewhere.[9] The tris-buffer solution (10 mM tris-HCl (PH 7.5) [(CH$_2$(OH))$_3$CNH$_2$], and 150 mM NaCl) are prepared and used immediately. Glycerol (99.9%) is purchased from Fisher Scientific

and used without further purification. The UV-Vis absorption spectra are recorded with CARY50 UV-Vis spectrophotometer (VARIAN). For emission spectra, the excitation light source is frequency doubled femtosecond Ti:sapphire laser, and the output wavelength is fixed at 430 nm. The fluorescence is collected by an optical fiber and introduced into charge coupled device (CCD) spectrometer (USB2000, Ocean optics). Picosecond temporally resolved spectra are obtained with a time-correlated single-photon counting (TCSPC) system (Fluotime 200, PicoQuant) as reported previously.[15]

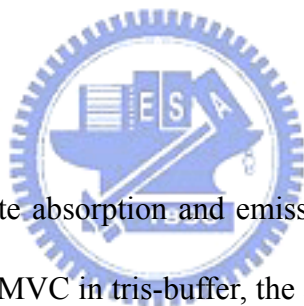## 5.3 Results and Discussion

5.3.1 Steady state spectra

Figure 5.1 is the steady state absorption and emission spectra of 5 μM BMVC in tris-buffer and glycerol. For BMVC in tris-buffer, the absorption band located at ~435 nm with a weak emission band at ~590 nm, and the total stoke shift is 6040 cm^{-1}. This result is similar to the result reported by Chang *et. al.*[11] To understand how intramolecular motion affects the excited state dynamics, we dissolved BMVC in glycerol. In glycerol, the intramolecular motion is suppressed due to its high viscosity (934 cp at 25 $^{\circ}$C). We noticed that the absorption band is red-shifted to 450 nm. The results can be explain in two ways. From the polarity point of view. The polarity of water is larger than that of glycerol ($\triangle$f=0.32 and 0.26 for water and glycerol, respectively), the observing bathochromic shift indicated that the dipole moment of

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[15] Bhongale, C. J., Chang, C.-W., Lee, C.-S. and Diau, E. W.-G. *J. Phys. Chem. B* **2005**, *109*, 13472-13478.

the excited state is small than that in ground state. Since the charge separation occurred between carbazole moiety and methyl pyridinium cation, it is reasonable for us to expect that the optical excitation will reduce the dipole moment of molecules. Second is from the structure point of view. Because the structure of BMVC is constructed by the repetition of C=C and C-C bond units, this red-shifted phenomena of the absorption spectra can be explained by the model of π-conjugated system, in which the absorption maximum would shift to long wavelength with the increasing of π-conjugation length.[16] Because the conjugation length relates with molecular geometry, this result indicates that the ground state geometry of BMVC in glycerol favors a planar structure. In glycerol, the fluoescence intensity increases dramatically, and the emission specra blue-shift to ~560 nm. The total stoke shift becomes 4370 cm^{-1}. This hypsochromic shift of the emission spectra and decrease of the Stoke shifts support that the intramolecular motion is restricted in glycerol.[10,11] Therefore, we expect that the enhancement of fluorescence intensity of BMVC in glycerol is due to the restriction of the molecular motion in high viscosity solvent.

5.3.2 Excited state dynamics of BMVC in tris-buffer

In order to get complete information about the excited state dynamics of BMVC in tris-buffer, we excite the molecules at 430 nm and probe the fluorescence decay within the range between 510nm ~640 nm. Figure 5.2A and Figure 5.2B are the typical fluorescence transients for BMVC in tris-buffer probing at 540 nm and 600 nm, respectively. Each transient is obtained by TCSPC and fluorescence

[16] Atkins, P. and Jones, L. *Chemical principles: The quest for insight*, 3; W. H. Freeman and company: New York, 2004

up-conversion technique (the data are shown in the inset). For the transients obtaining from TCSPC, at least three exponential decays are required to get a satisfactory fitting. The time-coefficient and relative amplitudes of each component are shown in Table, and the deconvolution of each component is shown in Appendix C. In Figure 5.2A, the data in the inset are obtained by fluorescence up-conversion technique, and the pulse-limited component is resolved due to its superior temporally resolution (~200 fs).
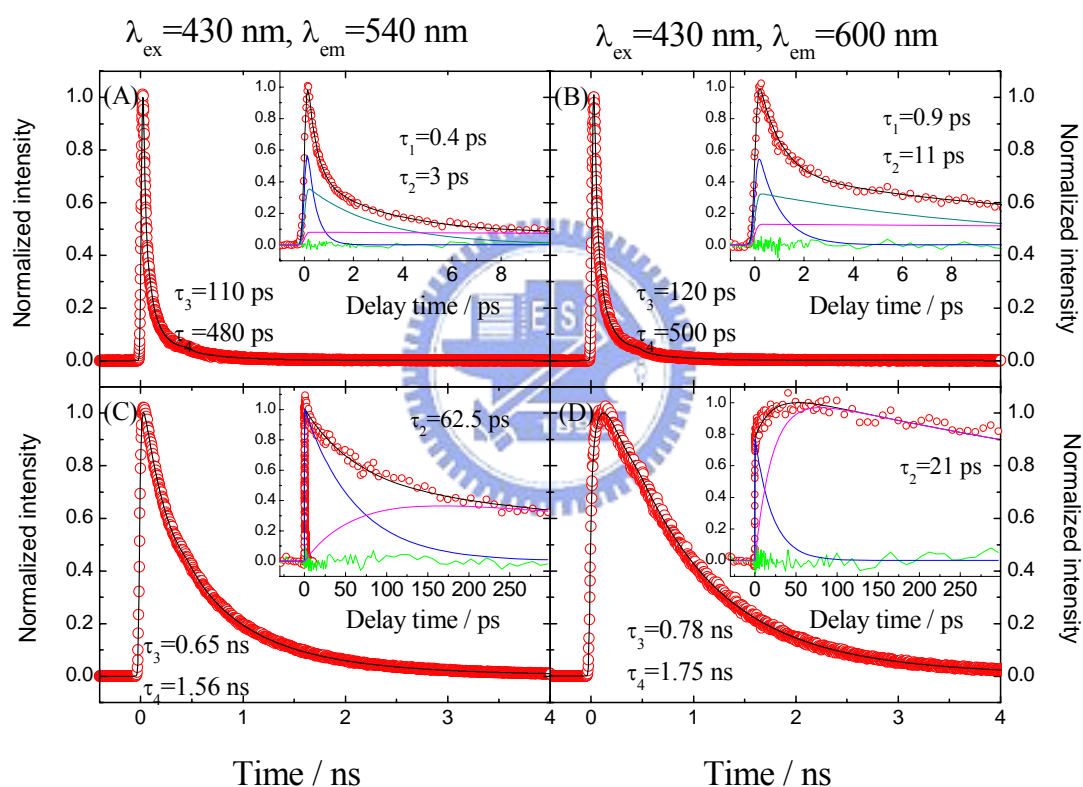

Figure 5.2: Time resolved fluorescence spectra of BMVC dissolved in tris-buffer and glycerol. The TCSPC result is shown in the main panel, and the up-conversion results in the inset. The fitting time-coefficients of each transient are shown as indicated. Figure A and B are the transients of BMVC in tris-buffer;Figure C and D are the transients of BMVC in glycerol.

Table: Fitting parameters of BMVC in tris-buffer, and glycerol. The value in brackets are the relative amplitude of each component. τ_3 and τ_4 was fixed at the value obtaining from TCSPC technique and indicated as blue color.

BMVC in tris buffer

Wavelength	τ_1	τ_2	τ_3	τ_4
510	0.3 ps (0.68)	1.3 ps (0.27)	90 ps (0.05)	440 ps
520	0.3 ps (0.67)	1.4 ps (0.28)	90 ps (0.05)	440 ps
530	0.4 ps (0.69)	2.5 ps (0.25)	100 ps (0.06)	460 ps
540	0.4 ps (0.65)	3.0ps (0.29)	110 ps (0.06)	480 ps
550	0.4 ps (0.67)	4.0 ps (0.26)	110 ps (0.07)	520 ps
560	0.6 ps (0.70)	6.5 ps (0.21)	120 ps (0.09)	520 ps
570	0.6 ps (0.74)	7.8 ps (0.20)	120 ps (0.06)	490 ps
580	0.8 ps (0.74)	14 ps (0.20)	120 ps (0.06)	510 ps
590	0.9 ps (0.66)	9.6 ps (0.25)	120 ps (0.09)	500 ps
600	0.9 ps (0.60)	11 ps (0.29)	120 ps (0.11)	500 ps
620	1.1 ps (0.54)	14 ps (0.33)	120 ps (0.13)	480 ps
640	1.4 ps (0.59)	12.0 ps (0.35)	130 ps (0.06)	480 ps

BMVC in glycerol

Wavelength	τ_1	τ_2	τ_3	τ_4
510				
520	1.2 ps (0.06)	36 ps (0.67)	510 ps (0.26)	1360 ps
530		44 ps (0.71)	590 ps (0.29)	1470 ps
540		62.5 ps (0.69)	650 ps (0.31)	1560 ps
550		82 ps (0.65)	650 ps (0.35)	1560 ps
560		152 ps (0.67)	730 ps (0.33)	1620 ps
570		127 ps (0.59)	630 ps (0.41)	1430 ps
580		5 ps (0.47)	710 ps (0.53)	1600 ps
590		15 ps (0.44)	750 ps (0.56)	1700 ps
600		21 ps (0.42)	780 ps (0.58)	1750 ps
620		28.0 ps (0.41)	790 ps (0.59)	1740 ps
640		37 ps (0.35)	800 ps (0.65)	1690 ps

For the transient obtained from up-conversion, it can be fitted with the following kinetic model:

$$I(t) = Amp1 \times e^{-t/\tau_1} + Amp2 \times e^{-t/\tau_2} + A3 \times (A_1 \times e^{-t/\tau_3} + A_2 \times e^{-t/\tau_4}) \qquad (1)$$

In this equation, A1, A2, τ_3, and τ_4 are fixed at the value of the corresponding TCSPC results, and used as a single parameter to describe the long component in the transient. For the transient probing at 510~530 nm, because of the interference of the third harmonic generation signal, an additional Gaussian is added to get the satisfactory fitting.

In our previous studies,[10] the excited state dynamics of 3,6-Distyrylcarbazole (3,6-DPVTCz) has been investigated. The structural similarity between BMVC and 3,6-DPVTCz indicates that the intrinsic process involving in the excited state of those two molecules should be similar. In 3,6-DPVTCz, both the twisting of CC single bond and CC double bond would lead to an efficient non-radiative process. The rotation of CC double bond has been extensively investigated in stilbene[17] and distyrylbenzene derivatives.[15] Those results reveal that it would lead to a barrier crossing internal conversion (IC) process. For above reason, the IC process should show a clear wavelength dependence feature. At long wavelength, the increasing of τ_1 and the decreasing of its relative amplitude strongly supported the above prediction. Therefore, τ_1 is attributed to the IC occurred via the rotation of CC double bond. After optical excitation, the excess vibration energy of the vibrationally hot molecules would dissipate to the surrounding environment. According to previous studies, the rate of vibration cooling process varied from ~ps[18,19] to tens of ps.[20,21] In general, vibration

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[17] Baskin, J. S., Banares, L. and Zewail, A. H. *J. Phys. Chem.* **1996**, *100*, 11920-11933.
[18] Kropman, M. F. and Bakker, H. J. *Chem. Phys. Lett.* **2003**, *370*, 741-746.

relaxation process is strongly depended on probing wavelengthes.[20,21] Both the time scale and wavelength dependent feature of τ_2 in agree with the above-mentioned feature, hence τ_2 is assigned to the vibration relaxation. The theoretical calculation results indicated that for 3,6 DPVTCz the C-C single bond rotation would lead to a barrier-less ISC process between S_1 and triplet state.[10] Therefore, the ~110 ps component and ~480 ps component is attributed to the ISC process. However, unlike the case in 3,6-DPVTCz, which only one time-coefficient is responsible for this process. We observed a bi-exponential decay in this case. This result indicated that there might have more than one minimum in S_1 state, which is similar, to what reported by Willets et al.[14] In their report, they proposed that there were two local minima in S_1 state, and each minimum was responsible for the individual lifetime.

In this section, the excited-state dynamics of BMVC in tris-buffer has been studied. Nevertheless, it should be noticed that the observing transients will also be affected by the solvation process between the solute and solvent molecules.[22] For above reason, it is worthwhile to reconstruct the solvation dynamics, and the result will be shown in next section.

5.3.3 Solvation dynamics of BMVC in tris-buffer

Because of the importance in biological systems, the hydration dynamics of solute in water has been extensively investigated in past decades. In general, the hydration dynamics of water is featured with a bi-exponential function. The ultrafast component

[19] Lock, A. J. and Bakker, H. J. *J. Chem. Phys* **2002**, *117*, 1708.

[20] Assmann, J., Benten, R. V., Charvat, A. and Abel, B. *J. Phys. Chem. A* **2003**, *107*, 5291.

[21] Tan, X., Gustafson, T. L., Lefumeux, C., Burdzinski, G., Buntinx, G. and Poizat, O. Ibid.**2002**, *106*, 3593.

[22] Glasbeek, M. and Zhang, H. *Chem. Rev.* **2004**, *104*, 1929.

(<200 fs) is attributed to the libration and intermolecular vibration; the slow (~ps) component is attributed to the diffusive rotation and translation.[23] In order to compare with the above-mentioned results, we reconstructed the time resolved emission spectra. Figure 5.3A are the femtosecond time-resolved fluorescence spectra of BMVC in tris-buffer, and the probing wavelength are changed from 510 nm to 640 nm with 10 nm interval. The emission spectra at various delay times are reconstructed by using the method proposed by M. Maroncelli and G. R. Fleming.[24] The reconstructed spectra are shown in Figure 5.4A. The result indicates that the spectra gradually shift from ~530 nm to ~600nm due to the stabilization of solvent molecules. In order to obtain better quantitative description of this time-dependent Stoke shift, the spectra are fitted with a log-normal equation,[24,25] and the average peak position at various time $\bar{\nu}(t)$ can be described with the following equation:

$$\bar{\nu}(t) = \bar{\nu}(\infty) + \left[\bar{\nu}(0) - \bar{\nu}(\infty) \right] \times \left(a_1 \times e^{-t/\tau_1} + a_2 \times e^{-t/\tau_1} \right) \tag{2}$$

In which $\bar{\nu}(0), \bar{\nu}(\infty)$, and $\bar{\nu}(0)$ indicate the averaged frequency at time-zero, time = infinite, and time = t, respectively. For comparison, above equation can be rearranged and defined as a solvation correlation function C(t):[24]

$$C(t) = \left[\bar{\nu}(t) \text{-} \bar{\nu}(\infty) \right] / \left[\bar{\nu}(0) - \bar{\nu}(\infty) \right] = a_1 \times e^{-t/\tau_1} + a_2 \times e^{-t/\tau_2} \tag{3}$$

In Figure 5.4B, a bi-exponential decay function is required to describe the C(t), and the time coefficients are 0.6 ps and 1.6 ps. According to previous results,[8,26,27] the 1.6 ps is attributed to the diffusive rotation or translation of water molecule. However, the

[23] Bagchi, B. *Annu. Rep. Prog. Chem, Sect. C* **2003**, *99*, 127.
[24] Maroncelli, M. and Fleming, G. R. *J. Chem. Phys* **1987**, *86*, 6221-6239.
[25] Siano, D. B. and Metzler, D. E. Ibid.**1969**, *51*, 1856-1861.
[26] Lu, W. Y., Kim, J. J., Qiu, W. H. and Zhong, D. P. *Chem. Phys. Lett.* **2004**, *388*, 120-126.
[27] Pal, S. K., Peon, J., Bagchi, B. and Zewail, A. H. *J. Phys. Chem. B* **2002**, *106*, 12376.

observing 0.6 ps component is significant longer than the corresponding component in bulk water. Dong-Ping Zhong group has reported the similar results,[26] in which the retardation of the ultrafast component is caused by the water molecules between solute and proximate solutes (tris-HCl).
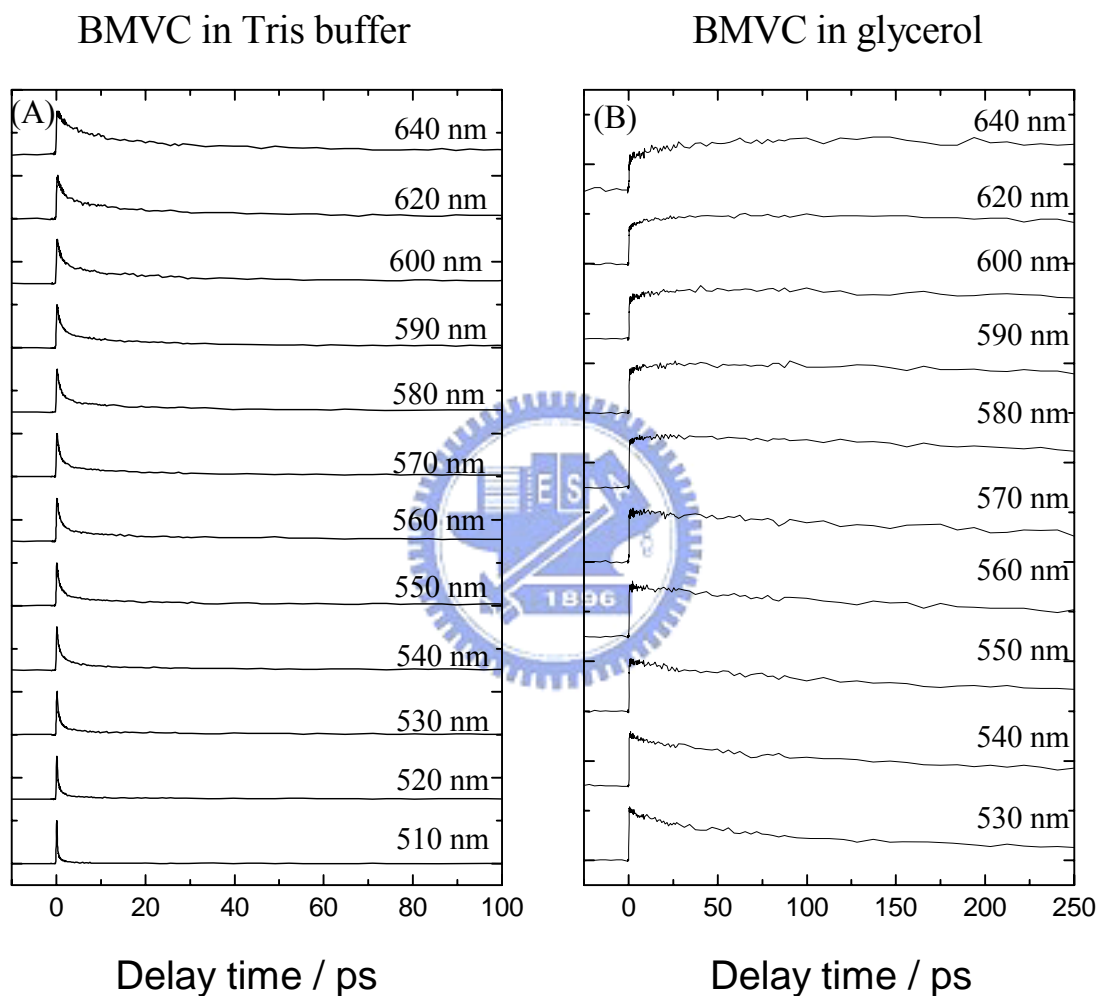
BMVC in Tris buffer   BMVC in glycerol

Figure 5.3: (A) Femtosecond time-resolved spectra of BMVC in tris-buffer with λ_{ex}=430 nm, and probing wavelength varied from 510 nm to 640 nm. (B) Femtosecond time resolved spectra of BMVC in glycerol with λ_{ex}=430 nm, and probing wavelength varied from 530 nm to 640 nm.
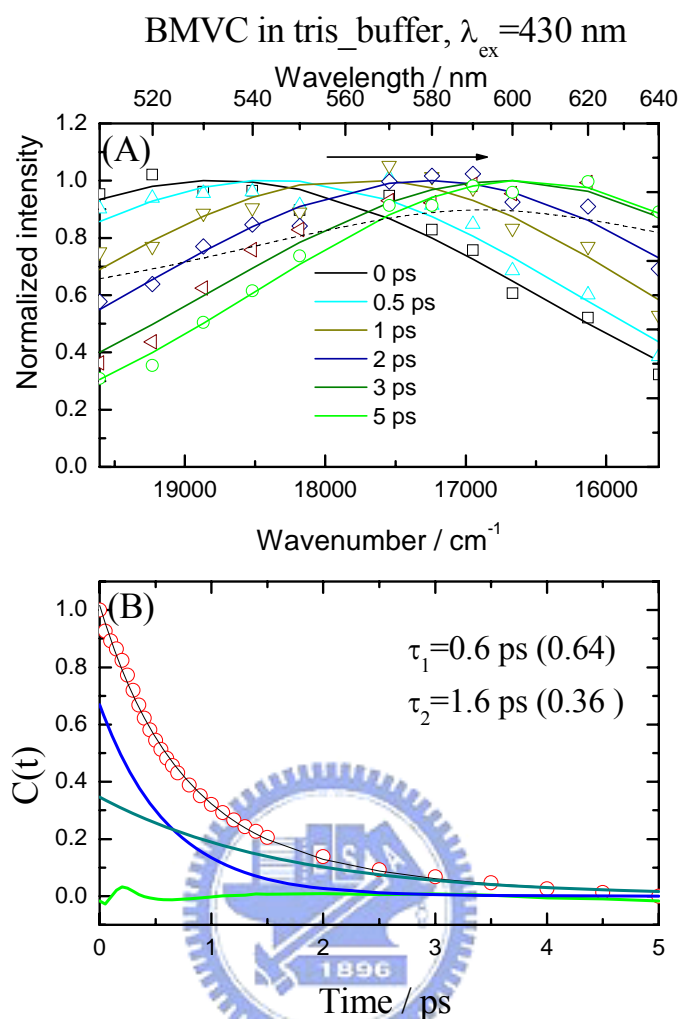
Figure 5.4: (A) The reconstructed time-resolved emission spectra of BMVC in tris-buffer. The row data at each delay time were shown as scatter and the fitted curves as solid curves. Steady state spectrum was indicated at dash line. (B) The C(t) value of BMVC in tris-buffer. Time coefficients were obtained by fitted the curve with bi-exponential model. The two components were indicated as blue and dark cyan curves, and the residual was indicated as green line.

5.3.4 Excited state dynamics and solvation dynamics of BMVC in glycerol

Figure 5.2C and Figure 5.2D are the typical fluorescence transients of BMVC in glycerol. The probing wavelength is fixed at 540 nm and 600 nm, respectively. In both wavelengths, the excitation wavelength is fixed at 430 nm. It is obvious that the fluorescence lifetime of BMVC in glycerol is longer than that in tris-buffer. This

result is in agreement with the steady state observation, in which the fluorescence intensity is enhanced in glycerol. In this case, the TCSPC results need to be fitted with three components. As the probing wavelengths varied from 540 nm to 600 nm, the initial decay part becomes a rise. The residual part of transient can be fitted with a bi-exponential function with time coefficient varied form 500~800 ps and 1.4 ns~1.8 ns. For up-conversion results (the transient in inset), the initial rise and decay feature are resolved, and the transients can be described with the following kinetic model:

$$A \xrightarrow{\tau_2} B \xrightarrow{\tau_3} C$$
$$A' \xrightarrow{\tau_2} B' \xrightarrow{\tau_4} C' \tag{4}$$
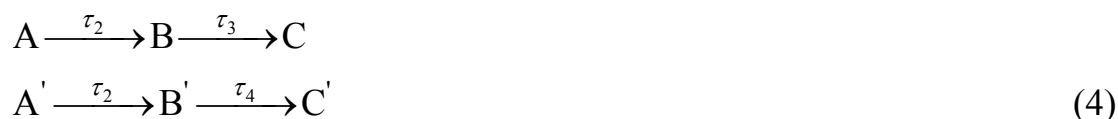
In this model, the relative amplitudes and time coefficients of τ_3 and τ_4 are fixed at the value obtained from TCSPC technique, and used to describe the slow decay and offset. A and A' indicate the BMVC with different geometries; B and B' represent the BMVC molecules at S_1 minimum. For BMVC in tris-buffer, the excited-molecule would be quenched via the rotational induced IC channel. In glycerol, this rotational motion would be restricted due to the high viscosity of glycerol, and the rate of IC would be decreased. However, we still observed an addition 1.2 ps component at 520 nm. Therefore, the 1.2 ps component at 520 nm is attributed to the IC conversion process. At the wavelength longer than 520 nm, because we probe the molecule with less available energy, the 1.2 ps component is absent. In the range between 520 nm and 570 nm, the observing τ_2 increases from 36 ps to 152 ps, and the relative amplitude decreases correspondingly. At the probing wavelength longer than 580 nm,

the probing window sets at the relative cold position, hence the decay component at

530~570 nm becomes a rise in this region. These rise and decay features are similar to

the general solvation phenomena. Finally, the observed τ_3 and τ_4 are one order of

magnitude longer than that in tris-buffer. In this case, the barrier of the molecule

rotation increases due the high viscosity. If the molecular rotation is totally suppressed,

the τ_3 and τ_4 can be expected as the radiative lifetime of BMVC with different

structures. Otherwise, τ_3 and τ_4 can be attributed to the rotational induced ISC process,

and it is reasonable for us to expect the retardation of those two processes in high

viscosity solvent.

In Figure 5.3B, we also show the time-resolved spectra of BMVC in glycerol, and

the probing wavelengths are varied from 530 nm to 640 nm. The reconstructed

emission spectra at various delay time are shown in Figure 5.5A, and the

corresponding C(t) value is shown in Figure 5.5B. The result in Figure 5.5A indicates

that the solvation process is not complete within the range between 0~100 ps.

Therefore, we also reconstructed the solvent response function C(t), by TCSPC

technique (data are shown in appendix D) and the 590 ps component in Figure 5.5B is

fixed at the value obtaining from TCSPC technique. In Figure 5.5B, the initial decay

of the C(t) is dominated by a ~18 ps decay, and the 590 ps component is shown as an

offset. According to previous results, the diffusive rotation component in solvation

process will be decelerated with the increasing of solvent viscosity.[28,29,30,31,32] For

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[28] Zhang, Y. and Berg, M. A. *J. Chem. Phys* **2001**, *115*, 4231.

[29] Simon, J. D. *Pure&Appl. Chem.* **1990**, *62*, 2243.

above reason, we expect that the observing 590 ps decay is due to the diffusive

rotation motion of glycerol molecules, and the 18 ps component is attributed to the

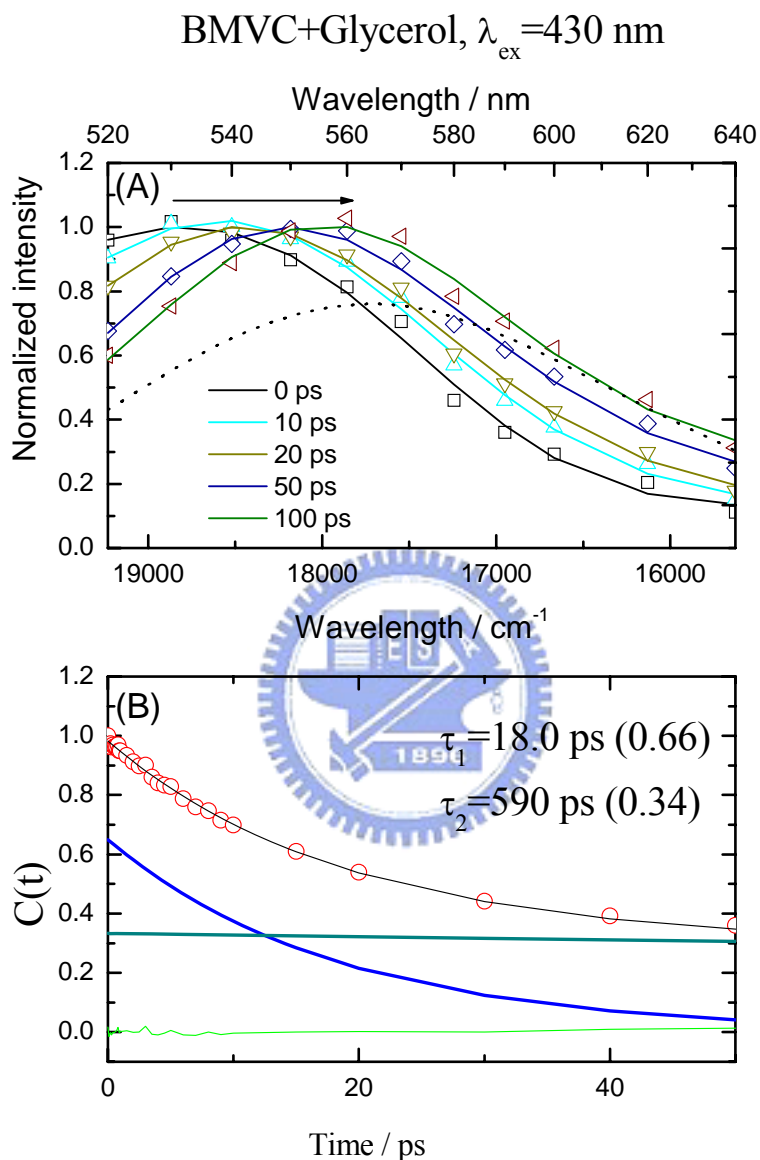local motion of the OH group or intermolecular vibration.

Figure 5.5: (A) The reconstructed time-resolved emission spectra of BMVC in glycerol. The row data at each delay time and the fitted curves were shown as scatter and solid curves, respectively. Steady state spectrum was indicated at dash line. (B) The C (t) value of BMVC in glycerol. The time coefficient was obtained by fitted the curve with bi-exponential model. The two components were indicated as blue and dark cyan curves, and the residual was indicated as green line.

[30] Zhang, Y., Jiang, J. and Berg, M. A. *J. Chem. Phys* **2003**, *118*, 7534.
[31] Zhang, Y., Sluch, M. I., Somoza, M. M. and Berg, M. A. Ibid.**2001**, *115*, 4212.
[32] Zhang, Y. and Berg, M. A. Ibid., 4223.

5.4 Concluding Remarks

In this study, the excited-state dynamics of BMVC in tris-buffer has been investigated. After initial excitation, a rotational induced IC competed with the solvent induced vibration relaxation. Because IC process is much faster (0.3~1.5 ps) than vibration relaxation (1.3 ps~12 ps), most of the excited-state populations are quenched by IC process. However, there still are small parts of molecules relaxing to S_1 minimum and returning to ground state via a rotational induced ISC channel with time coefficients of ~130 ps and ~480 ps. For BMVC in glycerol, the above-mentioned non-radiative process is suppressed by the high viscosity of glycerol. Therefore, the rate of rotational IC and ISC process is decreased, which results in the fluorescence enhancement we observed in steady state measurement. The solvation dynamic of BMVC in tris-buffer and glycerol are also studied. In tris-buffer, the biphasic nature of solvation correlation function is similar to the well-known solvation process in bulk water. However, because of the interaction between water molecules and tris-HCl, the observed dynamics (0.6 ps and 1.6 ps) is longer than that in bulk water. For BMVC in glycerol, the dramatic decrease of the solvation rate indicated that the high viscosity suppressed the rotation of the solvent molecule. The solvent-dependent solvation dynamics indicated that it could be used in probing the local environment of the solute molecules.