On the Doubly hydrogen bonded dimer of 7-azaindole (0.1 M) as a model for DNA base pairs in acetonitrile solutions at rt

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Multiple H-bonded base-pairing as a fundamental element of DNA structure was first described by Watson and Crick[1] using stable keto tautomer forms. In their analysis, they considered the possibility of mutations via tautomeric proton transfer shifts. Among other phenomena, such shifts can be caused by electronic excitation; for example, anomalous adenine-cytosine pairing may be a result of two-proton phototautomerism[2]. One suitable model base pair for two-proton translocating tautomerization is the C_{2h} dimer of 7-azaindole (7AI) proposed by Taylor *et al.*[3] in 1969. The most salient contribution of their work was that the double proton transfer in such a dimer occurs in a concerted manner. After the strong controversy raised in 1995 by the proposal of Douhal *et al.*[4] of a stepwise mechanism for the process was overcome, its concerted nature has been strongly supported by available evidence (see references 5-7 and references therein). Very recently, however, Kwon and Zewail [8] claimed to have obtained new supportive evidence that the proton phototransfer in 7AI dimer in polar solvents is in fact a stepwise rather than concerted

process. In this communication, we conducted a systematic spectroscopic study of the dimerization of 7AI at a 0.1 M concentration in acetonitrile that may allow us to demonstrate whether the 0.1 M 7AI solutions in acetonitrile at room temperature used by Kwon and Zewail[8] contained enough 7AI doubly hydrogen bonded dimer to enable its photoselection and hence its photophysical characterization with femtosecond / fluorescence spectroscopy.

To the author's knowledge, 7AI dimerization has been the subject of two studies in polar solvents that warrant discussion here. In one, Walmsley [9] found 7AI in dioxane at room temperature to form no dimers at concentrations as high as 0.5 M; in the other[10], the spectroscopic behavior of 7AI at a 10^{-4} M concentration in 1-chlorobutane from room temperature to low temperature values was characterized. The former study excluded the possibility of 0.1 M 7AI in ether occurring in dimeric form, as conceded by Kwon and Zewail[10]. The latter study provided a simple, accurate procedure for establishing whether 7AI dimerizes in polar solvents.

7-Azaindole H-bonded dimer results from the double hydrogen-bonding interaction between the corresponding pyrrole and pyridine units in two 7AI monomers, and is usually referred to as the C_{2h} dimer (see Scheme 1).

Scheme 1

Based on theoretical calculations at DFT level[11], the dimerization constant of 7AI increases markedly as the temperature is lowered; this is consistent with evidence for 7AI in 3MP obtained by Ingham and El-Bayoumi[12], who found the constant to increase from 1800 M^{-1} at 295 K to 10^6 M^{-1} at 200 K. When the first absorption band for the monomer and dimmer of 7AI are structured and the dimer is red-shifted from the monomer[1,10,13] (also Figures 6 and 7 of Supporting Information), it is easy to expose dimerization from absorption and emission spectra[10, 13, 14]. In this work we studied dimerization of 7AI at a 0.1 M concentration in acetonitrile by using a procedure involving lowering the temperature in a very gradual manner (about 0.1 K each 12 s) in order to maintain the solution in near-equilibrium conditions throughout the process.

Kwon and Zewail[8] reported two spectroscopic findings for 7AI in acetonitrile that warrant some comment here. One is that the first absorption band for 7AI at $1x10^{-5}$ and $1x10^{-2}$ M concentrations in acetonitrile as normalized to the maximum can be deemed identical inasmuch as they match each other (see Fig. 2 in ref. 8). In concluding that the more dilute solution contained monomer alone and the more concentrated solution contained a monomer/dimer mixture, Kwon and Zewail[8] implicitly assumed that the envelopes of the first absorption bands for 7AI dimer and monomer are identical, which, as shown below, is scientifically groundless.

Figure 1 reproduces the previous evidence with the bands for a $1x10^{-5}$ M solution of 7AI in acetonitrile (*i.e.* identical with one studied by Kwon and Zewail) and another for a much more concentrated solution (*viz.* $2x10^{-1}$ M, which is 20 times higher than that used by these authors). Coincidence between the two spectral envelopes is so strong that one can easily be inclined to believe that both bands are produced by the same species.

Figure 1

If, as assumed by Kwon and Zewail[8], both absorption bands match, then 7AI monomer in acetonitrile should undergo an unexpected red shift offsetting that resulting from 7AI dimerization, which amounts to *ca.* -600 cm^{-1}, since the distance between the absorption maxima for the monomer and dimer is approximately -713 cm^{-1} in 3MP[3], -598 cm^{-1} in 2MB[13], -598 cm^{-1} in heptane, -655 cm^{-1} in decalin and -537 cm^{-1} in 1-chlorobutane[10]. Table 1 shows the wavelengths of the measured maxima for the monomer and doubly hydrogen bonded dimer of 7AI in various solvents. As can be seen, the maximum for the dimer is at 292.2 ± 1 nm and that for the monomer at 287 ± 0.5 nm (*i.e.* they are highly insensitive to the nature of the particular solvent). Also, the absorption maximum for a $1x10^{-5}$ M solution of 7AI in ACN is at 287.5 nm and thus quite consistent with the previous monomer value. Because 7AI dimer is non-polar, non-acidic and non-basic, it can only be sensitive to the polarizability of ACN, which is in between those of 2MB and decalin. Therefore, the dimer absorption maximum should be at *ca.* 292 nm and, if the absorption band for 0.1 M 7AI in ACN matches that for a $1x10^{-5}$ M solution, then both must contain the same species and such species can only be the monomer.

In Fig. 2 of their paper, Kwon and Zewail (8) show the fluorescence bands as normalized to the maximum –which lies at *ca.* 360 nm– for a $1x10^{-5}$ M solution excited at 290 nm, and two containing 0.05 M and 0.1 M 7AI, respectively, excited at 320 nm. In their figure, the most concentrated solution (0.1 M) exhibits an emission band at *ca.* 500 nm which they assigned to 7AI dimer. Our Fig. 2 shows in a similar manner the emission bands for a $1x10^{-5}$ M solution and a $1x10^{-1}$ M solution of 7AI in acetonitrile but excited at 320 nm in both cases. As can be seen, no emission from 7AI dimer is apparent; in fact, only a slight red shift in the spectral envelope for the more concentrated solution relative to the less concentrated one ($1x10^{-5}$ M) is observed.

Figure 2

Because neither the absorption nor the emission rt spectra was consistent with the presence of a 7AI dimer, the temperature of the 0.1 M solution was lowered in order to amplify the potential dimerization process to an extent facilitating detection of any dimer potentially present.

Figure 3

The absorption spectra for 0.1 M 7AI in ACN at temperatures from room temperature to the melting point of ACN (225 K), shown in the inset of Fig. 4, provide no evidence for the presence of dimer. Figure 3 shows the emission spectra for a 0.1 M solution of 7AI in ACN at 275, 255 and 235 K as obtained with excitation at 320 nm; as can be seen, they exhibit no band at 500 nm, where 7AI dimer typically emits. Since Kwon and Zewail[8] conducted their femtosecond spectroscopic tests with excitation at 313 nm, we also recorded spectra at that excitation wavelength (see Figure 8 of Supporting Information). As expected, such spectra provided no evidence for the presence of the dimer. This was further confirmed by the excitation spectra obtained by monitoring light at 500 nm (see Figure 9 of Supporting Information), which were clearly due to the 7AI species present in ACN: the monomer.

Figure 4

Figure 4 shows the absorption spectra obtained at wavelengths above 290 nm for a 0.1 M solution of 7AI in butyronitrile at 20 K intervals over the temperature range 295-135 K. The spectra warrant some interesting comments. Thus, the butyronitrile matrix allows spectra of adequate optical quality to be

obtained even at temperatures below its melting point. Also, the spectra obtained between 295 and 235 K are similar to those recorded in ACN (see inset of Fig. 4), which are suggestive of the absence of dimerization in these solutions. Finally, the spectra recorded below 195 K exhibit a substantial red shift clearly suggesting that a new chromophore is being produced; therefore, the assumption of Kwon and Zewail[8] that the spectral envelopes of the first absorption bands for 7AI monomer and dimer in ACN are identical is incorrect.

Figure 5 shows the emission spectra for the previous solution at temperatures from 295 to 135 K as obtained with excitation at 320 nm. A new emission band in the region of 500 nm is now clearly observed that increases as the temperature is lowered. The corresponding excitation spectra obtained by monitoring light at 500 nm, which are gathered in Figure 10 of Supporting Information, clearly reveal the presence of a new species at temperatures below 195 K; such a species is the dimeric form of 7AI.

In summary, it seems obvious that only below 238 K 7AI exhibits dimmer formation in nitrile solvents. We can therefore unequivocally state that the 0.1 M 7AI solution in acetonitrile at room temperature studied by Kwon and Zewail contained no electronically excitable dimeric form and hence that their conclusions reached with the aid of femtosecond spectroscopy cannot apply to 7AI dimer.

Figure 5

Equivalent experiments undertaken in our laboratory using 0.1 M solutions of 7AI in ethyl ether and dichloromethane, which are the other polar solvents used by Kown and Zewail[8], foster the conclusion of that the 7AI dimer species is not formed in these solutions at rt.

In summary, the assumption of Kwon and Zewail[8] that they were exciting doubly hydrogen bonded dimers of 7AI at room temperature in these solvents was incorrect. If 7AI solutions in diethyl ether, dichloromethane and acetonitrile contain no doubly hydrogen bonded dimers, then the arguments of Kwon and Zewail[8] in support of an stepwise double proton transfer mechanism are groundless and the original proposal that the C_{2h} dimers of 7AI undergo concerted double proton transfer upon excitation remains unchallenged.

Experimental Section

7-Azaindole was obtained from Sigma in 99% purity and recrystallized twice in spectroscopic-grade cyclohexane. All 7AI solutions in acetonitrile (Merck Uvasol grade) and butyronitrile (Aldrich 99% pure or better) contained a concentration of 0.1 or 0.2 M of the compound. The sample temperature ranged from 138 to 295 K and was controlled by using an Oxford DN1704 cryostat equipped with an ITC4 controller interfaced to the spectrophotometers. UV-Vis spectra were recorded on a Cary-5 spectrophotometer. Corrected fluorescence and fluorescence excitation spectra were obtained on a calibrated Aminco-Bowman AB2 spectrofluorimeter. A thorough experimental Section has been implemented in the Supporting Information.

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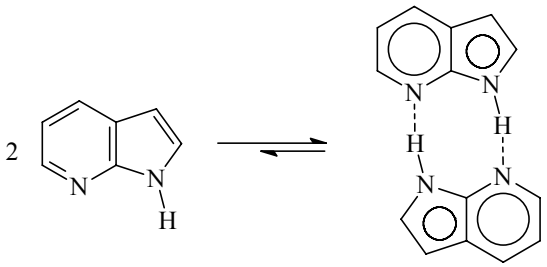

C_{2h}

Scheme I Dimerization process

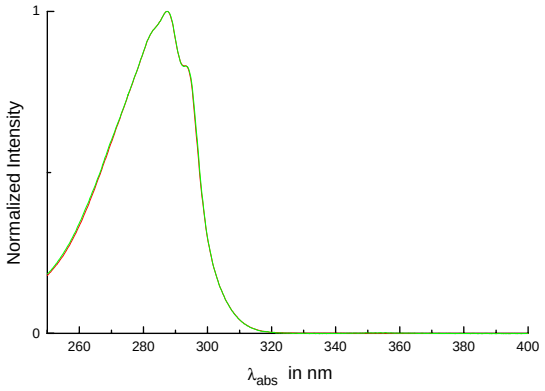

Figure 1 First absorption band of 7AI 1 x 10^{-5} M (green) and 0.2 M (red)) solutions in ACN at 295 K; normalized at the maxima.

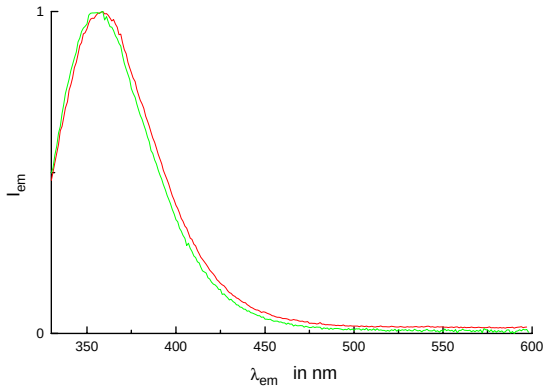

Figure 2 Emission spectra normalized at the maxima of 7AI (1 x 10^{-5} M(green) and 0.1 M(red)) solutions in ACN , on excitation at 320 nm.

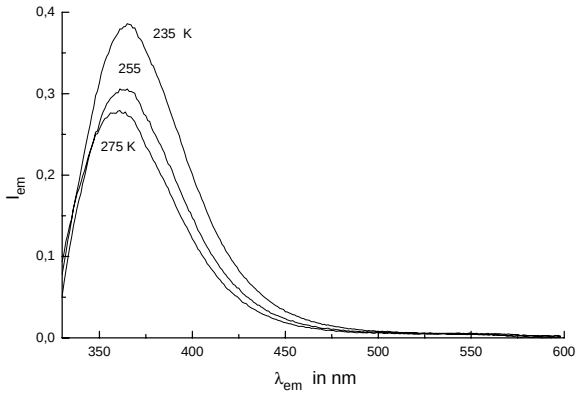

Figure 3 Emission spectra of 7AI (0.1 M) in ACN at 275, 255, and 235 K; on excitation at 320 nm.

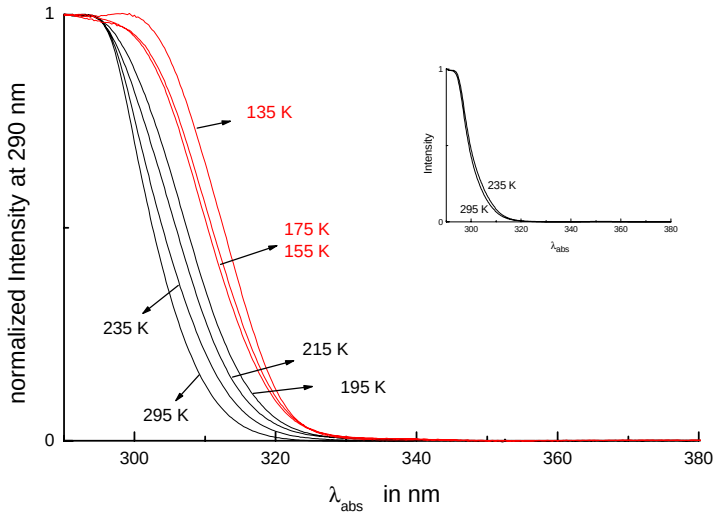

Figure 4 Absorption spectra, normalized at 290 nm, recorded between 290 and 380 nm for 7AI (0.1 M) in BuCN from 295 K to 135 K. The inset shows the corresponding absorption spectra for 7AI (0.1 M) in ACN at 295 K and 235 K.

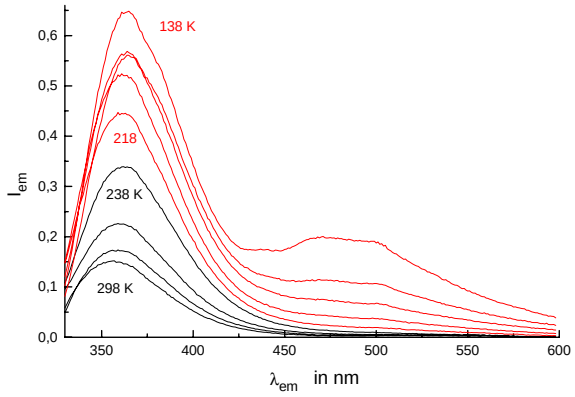

Figure 5 Emission spectra from 295 K to 135 K for 7AI (0.1 M) in BuCN on excitation at 320 nm.

Table 1. Wavelengths for the absorption maxima of the 7AI monomer and dimer in several solvents.

Solvent	λ^{max} (dimer) in nm	λ^{max} (monomer) in nm
2-methylbutane (2MB)	291.8	286.8
n-heptane	291.8	286.8
3-methylpentane (3MP)	293.2	287.2
Decaline	292.5	287.0
1-Chlorobutane	291.8	287.3
Acetonitrile (ACN)	------	287.5

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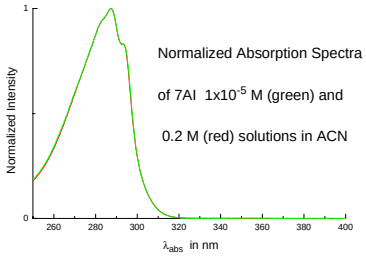

Supporting Information Available

Experimental Section

7-Azaindole was obtained from Sigma in 99% purity and recrystallized twice in spectroscopic-grade cyclohexane. All 7AI solutions contained a concentration of 0.1 or 0.2 M of the compound in various solvents that were all purchased in the highest available purity. Thus, acetonitrile was Merck Uvasol grade and butyronitrile Aldrich 99% pure or better. The sample temperature ranged from 138 to 295 K and was controlled by using an Oxford DN1704 cryostat equipped with an ITC4 controller interfaced to the spectrophotometers. The cryostat was purged with dried nitrogen 99.99% pure.

UV-Vis spectra were recorded on a Cary-5 spectrophotometer, using a cell of *ca.* 10 μm light path at room temperature and a Suprasil cylindrical cell of 1 mm light path mounted on the cryostat at variable temperatures. All spectroscopic emission measurements were made in Suprasil cylindrical cells of 1 mm light path; as a result, the path length to the cell, which governs so-called "filtering effects" on fluorescence –a major factor with highly absorbing solutions– was less than 0.5 mm (the average path length ranged from 0 to 0.5 mm). All 7AI samples were excited by using light from a continuous wave (CW) 150 W xenon lamp.

Corrected fluorescence and fluorescence excitation spectra were obtained on a calibrated Aminco-Bowman AB2 spectrofluorimeter. The sensitivity factors for the emission channel of the spectrofluorimeter, which include not only those depending on the detector but also those depending on the emission monochromator and optical arrangement (including the channel emission), were obtained by using the FP-123 correction kit from SLM Instruments, Inc. The kit was used to mount a standard lamp in a channel at right angles from the emission channel of an OL 245 M spectral irradiance lamp from Optronic Laboratories, Inc. The standard lamp was operated at a constant voltage provided by an SP-720 power supply. The light generated by the lamp was conducted into an integrating sphere possessing a pinhole exit to conduct the light to the emission channel of the fluorimeter. The conversion

factors thus obtained allowed the technical spectra to be transformed into absolute spectra, which are independent of the particular spectrophotometer used.

Corrected excitation spectra were obtained directly with the AB2 spectrophotometer; a small fraction of the light intensity for excitation was switched, by using a beam splitter, to a Hamamatsu S1336-8BQ photodiode the photosensitivity *vs*. wavelength curve for which allowed changes in incident light intensity at each excitation wavelength to be detected. The ratio of the emission intensity at the monitored wavelength to the corresponding excitation intensity at each excitation wavelength provided the absolute excitation spectrum.

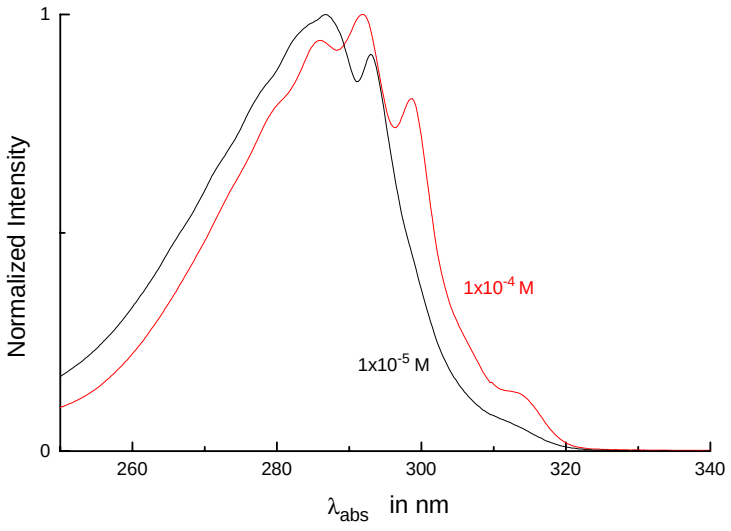

Figure 6 Absorption spectra for 7AI monomer and dimer in n-heptane, normalized at the maxima. The monomer spectrum corresponds to a 10^{-6} M solution of 7AI, in which monomer is mostly the species present; and the dimer spectrum corresponds to a 10^{-4} M solution of 7AI at low temperature, in which only dimer is present.

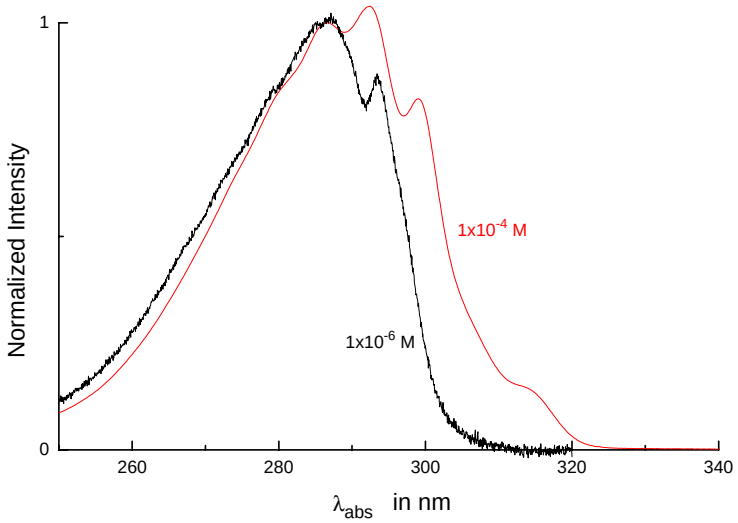

Figure 7 Absorption spectra for 7AI monomer and dimer in decaline, normalized at the maxima. The monomer spectrum corresponds to a 10^{-6} M solution of 7AI, in which only monomer is present; and the dimer spectrum corresponds to a 10^{-4} M solution of 7AI at low temperature, in which only dimer is present.

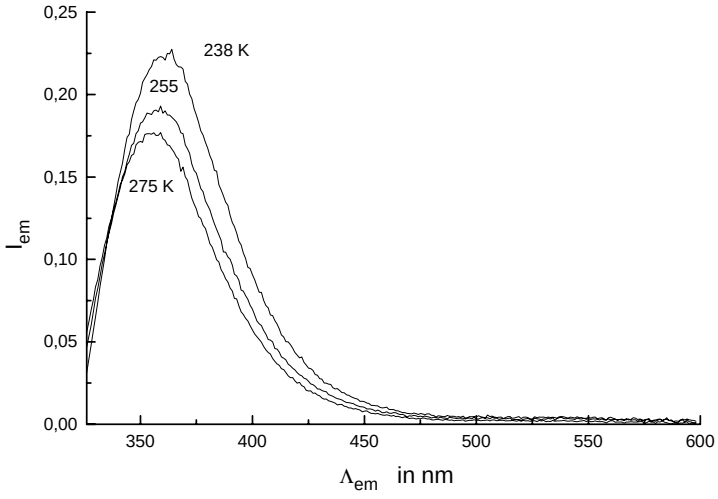

Figure 8 Emission spectra of 7AI (0.1 M) in ACN at 275, 255, and 235 K; on excitation at 313 nm.

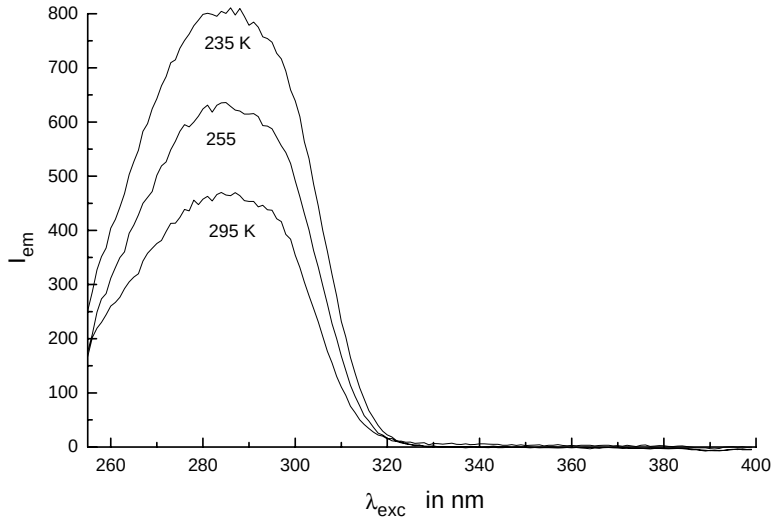

Figure 9 Excitation spectra of 7AI (0.1 M) in ACN at 275, 255, and 235 K; recorded by monitoring light emission at 500 nm.

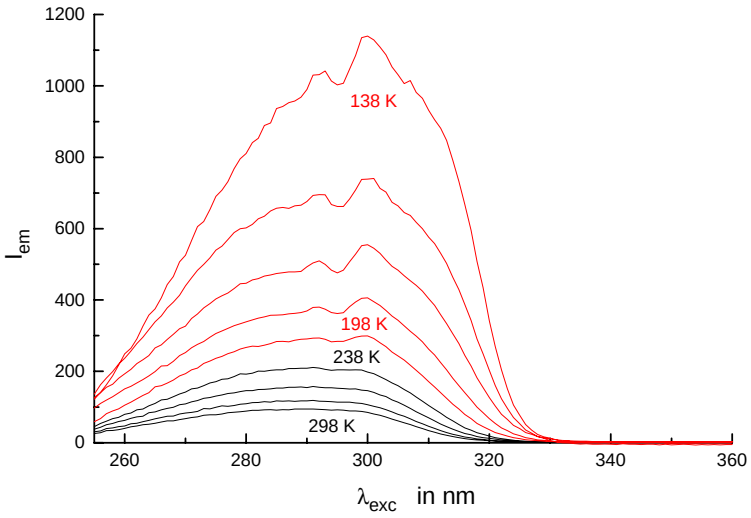

Figure 10 Excitation spectra from 295 K to 135 K for 7AI (0.1 M) in BuCN recorded by monitoring light emission at 500 nm.