

## Reply to "Comment on 'An Interesting Case Where Water Behaves as a Unique Solvent. 4-Aminophthalimide Emission Profile to Monitor Aqueous Environment'"

Andrés M. Durantini,<sup>†</sup> R. Darío Falcone,<sup>†</sup> Jorge D. Anunziata,<sup>†</sup> Juana J. Silber,<sup>†</sup> Elsa B. Abuin,<sup>‡,§</sup> Eduardo A. Lissi,<sup>‡</sup> and N. Mariano Correa<sup>\*,†</sup>

<sup>†</sup>Departamento de Química, Universidad Nacional de Río Cuarto, Agencia Postal # 3, (X5804ALH) Río Cuarto, Argentina

<sup>‡</sup>Universidad de Santiago de Chile (USACH), Casilla 40- Correo 33, Santiago, Chile

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In a recent paper<sup>1</sup> the behavior of 4-aminophthalimide (4-AP), a common molecular probe utilized in solvation dynamics experiments, was revisited in polar aprotic and protic solvents using absorption, steady-state, and time-resolved fluorescence (TRES) techniques. Also, the deuterium isotope effect was investigated using D<sub>2</sub>O as solvent.

We found that in accordance with literature the absorption spectra of 4-AP consist of two absorption bands with maxima around 300 (B<sub>2</sub> band) and 370 nm (B<sub>1</sub> band) depending on the solvents properties, while the emission feature is a single band that also shows solvatochromism. We showed that in all solvents investigated (*excluding water*) the 4-AP photophysics is similar in the sense that the emission spectra (*steady-state experiments*) are independent of the excitation wavelength used. However, in water, the behavior is unique and the emission spectra maxima are different depending on the excitation wavelength used. The emission maximum wavelength is 561.7 nm upon excitation on the B<sub>2</sub> band ( $\lambda_{\text{excB}_2} = 303.4$  nm). When the excitation wavelength used corresponds to the B<sub>1</sub> band ( $\lambda_{\text{excB}_1} = 370.0$  nm) the emission maximum wavelength shifts to 545.7 nm.

To obtain insights into those peculiar results we performed TRES<sup>2</sup> measurements on 4-AP dissolved in different organic solvents and water, not only monitoring at different emission wavelengths but also exciting at different wavelengths. We showed that while the 4-AP emission decay in water exhibits no emission wavelength dependence at  $\lambda_{\text{excB}_2}$ , when  $\lambda_{\text{excB}_1}$  was used time-dependent emission spectra that shift to the blue with time were found. To explain the 4-AP behavior, we proposed a mechanism that tries to account for the steady-state and the time-resolved results in all organic solvents and water. Our results show that the solvent-mediated proton-transfer process displays a fundamental role in the 4-AP emission profile, and, for the first time, the proposed mechanism explains the 4-AP behavior in every medium. The deuterium isotope effect confirms the importance of the proton-transfer process in water, being dramatically retarded in deuterated water. No emission maxima dependence with either the excitation wavelength or with time was found.

Samanta et al.<sup>3</sup> have written a Comment on our work where they have criticized our results and the mechanism used to explain them. In the Comment, they showed a figure where the

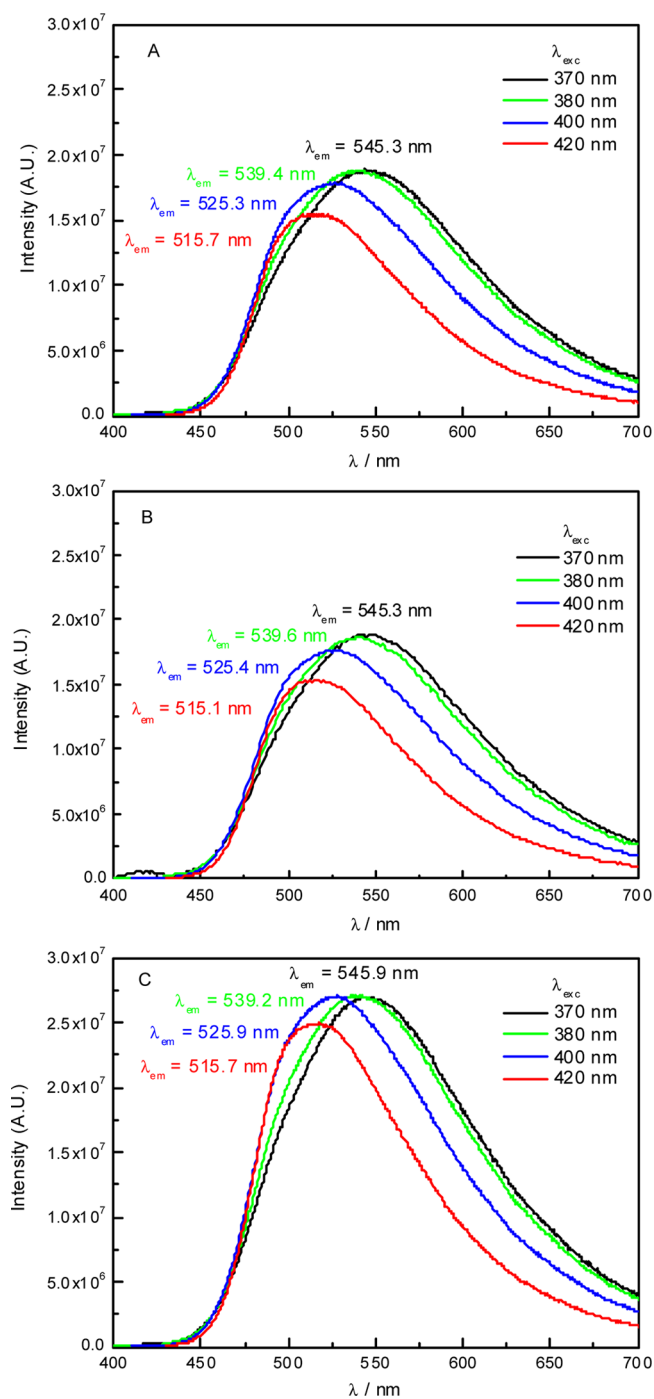
4-AP emission spectra position (*steady-state experiment*) does not depend on the excitation wavelength used. Unfortunately, the plots in the figure are normalized and much valuable information is lost. We present Figure 1A–C that shows the 4-AP emission maxima dependence with the excitation wavelength that we have obtained for  $[4\text{-AP}] = 6 \times 10^{-5}$  M (Sigma or Across Organics 97%) as received and purified by double sublimation and for  $[4\text{-AP}] = 1 \times 10^{-4}$  M. As it can be seen, we found again a clear dependence of the 4-AP emission maxima on the excitation wavelength at the red edge of the B<sub>1</sub> band, even at different dye concentration. Samanta et al. suggested that we probably have an instrument problem (bad calibration or bad fluorescence correction file) that causes artifacts in our data. It is known that when an instrument has those kinds of problems, all of the samples show artifacts. Our work<sup>1</sup> clearly shows that in all of the organic solvents used (except water), the emission feature is that expected: there is no excitation wavelength dependence on 4-AP emission spectra at both absorption B<sub>1</sub> and B<sub>2</sub> bands. Thus, those results discard the possibility of artifacts from the instruments. Another possibility that has not been taken into account by Samanta et al. and can be the cause of the differences observed between both groups is the way that 4-AP is introduced in water to prepare the solution. This is not trivial when diluted solutions are prepared using molecular probes with low solubility. The way that our water solution was prepared was described in detail in the work.<sup>1</sup> Also, special care has to be taken when 4-AP is purified by crystallizing it from ethanol. It is hard to completely eliminate the solvent because it tends to be occluded in 4-AP structure. To avoid that, we purified 4-AP by double sublimation.

Samanta et al. also argued that we have not tried to compare our emission data with those reported in the literature for fixed excitation wavelengths. We want to highlight that in most of the works in the literature it is extremely difficult to find spectroscopic data like excitation wavelengths used or 4-AP emission maxima values. In the majority of the cases, they have

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**Figure 1.** 4-AP emission spectra in water at different excitation wavelengths. (A) [4-AP] =  $6 \times 10^{-5}$  M (Acros Organics or Sigma 97%). (B) [4-AP] =  $6 \times 10^{-5}$  M (purified by double sublimation). (C) [4-AP] =  $1 \times 10^{-4}$  M (Acros Organics 97%).

to be supposed (by reading the work) or estimated from figures in the paper. Nevertheless, in our work, there is a detailed discussion of different works.<sup>1</sup> Samanta et al. pointed out the data obtained from Dobek's group<sup>4,5</sup> to show that the 4-AP emission band seems to be independent of the excitation wavelength. We present the following data obtained from the literature that were properly referenced in our work. The group of Bhattacharyya et al. showed that for  $\lambda_{\text{exc}} = 369$  nm ( $\lambda_{\text{excB1}}$ ), 4-AP emits at  $\lambda_{\text{em}} = 540$  nm,<sup>6</sup> whereas, in other work of the same group, when the excitation wavelength used was  $\lambda_{\text{exc}} = 300$  nm

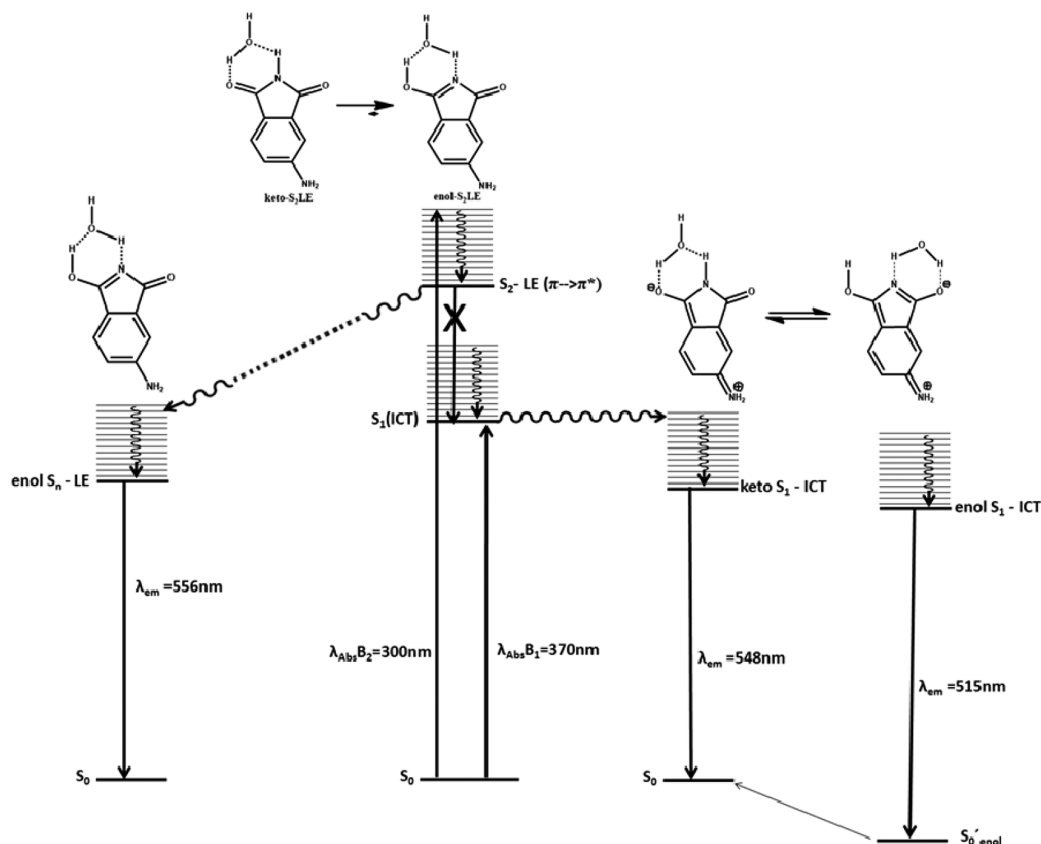
( $\lambda_{\text{excB2}}$ ), the emission maximum value was  $\lambda_{\text{em}} \approx 560$  nm.<sup>7,8</sup> Even more, Dobek et al. showed that at  $\lambda_{\text{exc}} = 369$  nm ( $\lambda_{\text{excB1}}$ ), 4-AP emits at  $\lambda_{\text{em}} = 560$  nm,<sup>9</sup> whereas Noukakis et al. showed that at  $\lambda_{\text{exc}} = 360$  nm ( $\lambda_{\text{excB1}}$ ), 4-AP emits at  $\lambda_{\text{em}} \approx 545$  nm (obtained through figure 1b of the reference).<sup>10</sup> The different data presented in the literature show that the 4-AP emission band is wavelength-dependent in concordance with our results. Thus, it seems to us that the 4-AP photophysics is far from being completely understood.

We want to remark that one important point that Samanta et al. have completely omitted in their Comment is the complete and thorough investigation using TRES experiments that we show in the work,<sup>1</sup> data that are obtained in a completely different instrument than the one used to obtain the steady-state emission spectra. Those results were also unusual and showed that, in water, the 4-AP emission band shifts to the blue with time *only* if the excitation wavelength is the one that corresponds to  $\lambda_{\text{exc}} = 370$  nm ( $\lambda_{\text{excB1}}$ ).

With regard to the mechanism proposed to explain our intriguing results that Samantha et al. also criticized, we highlight the fact that the mechanism was thought to explain the steady-state and the time-resolved results in all solvents used, including water. It is absolutely debatable and probably can be improved if molecular modeling and computer simulation on 4-AP behavior is performed.

In the Comment, they said that the mechanism is unrealistic and inconsistent with our data, but we will show that those statements are not valid. The main objection on the mechanism is that the scheme 2 shows that 4-AP in the ground state exists in both keto and enol forms in equilibrium, in aqueous solution. If this is the case, then we would make direct excitation of the enol forms and monitor the direct emission from it. Let us say that never in the text of the work<sup>1</sup> did we say that there is a keto–enol equilibrium in the 4-AP ground state. On the contrary, we propose that only one 4-AP species exists in the ground state: the keto species. Unfortunately, scheme 2C<sup>1</sup> shows a double arrow in the ground state, which is a typographical mistake, and we sent the proper correction to the *Journal of Physical Chemistry B*. (See the correct scheme 2C in the present Reply (Scheme 1).) The key of our explanation summarized in the Scheme is that the 4-AP excited states are different if the molecule is excited at  $\lambda_{\text{exc}} = 300$  nm ( $B_2$ ), where the locally excited (LE) state is populated, than if the excitation is at  $\lambda_{\text{exc}} = 370$  nm ( $B_1$ ), where the intramolecular charge transfer (ICT) state is populated. In all of the solvents, except water, the emission always comes from the same excited state, the keto ICT state, and the emission spectra do not present dependence on the excitation wavelength. In water, the situation seems to be different because when the LE state is populated ( $\lambda_{\text{exc}} = B_2$ ) the fast conversion to the enol LE species makes the formation of the keto ICT state impossible and the emission comes from the enol LE state. When the ICT state was populated ( $\lambda_{\text{exc}} = B_2$ ), we proposed a keto–enol equilibrium in the 4-AP excited state for the ICT species. The emission goes to different ground states,  $S_0$  for the keto species and  $S_0'$  for the enol species, because the different 4-AP electronic charge distributions of the keto and enol ICT species are not equally solvated by water. Also, we assumed that  $S_0'$  is unstable with a short lifetime and rapidly converts to the most stable ground-state configuration,  $S_0$ .

Finally, we would like to stimulate the scientific community to further investigate this fascinating molecular probe to put light on its controversial photophysics.

Scheme 1. Emission Mechanism and the Solvent-Mediated Proton Transfer for 4-AP in Water at Any Excitation Wavelength<sup>a</sup>

<sup>a</sup>S<sub>0</sub>' represents the unstable with a short lifetime enol-ICT ground-state species that rapidly converts to the most stable ground state, S<sub>0</sub>.

## AUTHOR INFORMATION

### Corresponding Author

\*E-mail: mcorrea@exa.unrc.edu.ar. Tel: 54-358-4676233.

### Notes

The authors declare no competing financial interest.

## DEDICATION

§Dedicated to the memory of Prof. Dr. Elsa Abuin who died on April 18, 2012

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