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Comment on "Binding of alkaloid harmalol to DNA: Photophysical and calorimetric approach"

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ABSTRACT

The present manuscript is a comment on the article entitled "*Binding of alkaloid harmalol to DNA: Photophysical and calorimetric approach*" by Sarita Sarkar and Kakali Bhadra (2014) [1]. In their article, the authors reported the chemical structure as well as the absorption spectra of harmalol at different pH. On the bases of the previous publications and our own present results, the assignment of the number of the acid-base species and the corresponding pK_a values, as well as the chemical structure for each species are erroneous. These facts have strong effect on the conclusions reached by the authors, in terms of the interaction with DNA.

1. Results and discussion

In their article (Sarita Sarkar and Kakali Bhadra, *Journal of Photochemistry and Photobiology B: Biology*, 2014, 130, 272–280) [1], Sarkar and Bhadra reported the chemical structure as well as the absorption spectra of harmalol at different pH. The authors stated that in the pH range 1–12 only one acid-base equilibrium occurs between the two species depicted in Fig. 1. They also inform that the pK_a value in water is 7.8.

Harmalol belongs to a family of alkaloids called β-carbolines (βCs). βCs comprise both fully aromatic alkaloids and partially reduced derivatives such as 3,4-dihydro- and 1,2,3,4-tetrahydro-βCs. In terms of its chemical structure, harmalol is a 3,4-dihydro-βC.

In aqueous solution, these alkaloids may show several acid–base equilibria. The latter fact was reported several decades ago, [2–6] but these publications are not cited by Sarkar and Bhadra. In these former studies, it was established that the general trend for βCs is as follows: in the pH range 3–11, there is only one acid-base equilibrium that involves the protonation/deprotonation of the pyridinic nitrogen (N2), with a pK_a value ~7 and ~9 for fully aromatic and 3,4-dihydro-βCs, respectively (Fig. 2). It is worth mentioning that, the deprotonation of both the indolic nitrogen (N9) and also the hydroxylic group (located at C7) are expected to occur only under extremely alkaline conditions. [5] Moreover, in the particular case of harmalol, the protonation of the hydroxylic group would also take place under extremely acidic conditions (pH <2). Therefore, the chemical structures shown by Sarkar and Bhadra in Fig. 1 are incorrect.

The absence of some kind of explanation by Sarkar and Bhadra about the quite high difference between the newly reported value (7.8) [1] and the expected one (~9), together with the fact that no other acidic and/or basic species were observed in the pH range investigated by these authors incited us to reproduce our own titration measurements (Fig. 3) and to determine again the pK_a values for harmalol in the pH range 1–12. It is worth mentioning that Fig. S1 provided by Sarkar and Bhadra clearly shows the absence of isosbestic points in the whole pH range investigated. This fact is indicative of the presence of other acid-base equilibria that were not detected by the authors.

We found two different equilibria in the pH-range investigated (Fig. 4): (i) one observed between pH 3 and 10 that was assigned to the protonated and neutral species (*i.e.*, forms a and b, respectively), with a pK_{a1} value of 8.5 and (ii) another one assigned to the neutral and the mono-anionic species (forms b and c, respectively), with a pK_{a2} value of 11.2. These two pK_a values are consistent with those published by Vert's et. al. [7] in 1984 (i.e., pK_{a1} and pK_{a2} of 8.6 and 11.5, respectively). The corresponding absorption spectra are shown in Fig. 3d. Spectra of harmalol buffer solutions are in good agreement with those recorded in aqueous solutions (results not shown).

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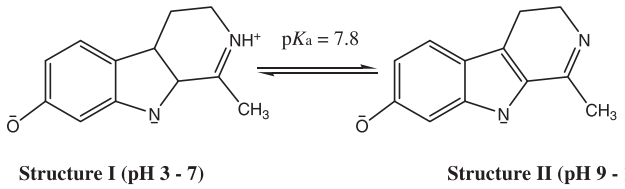

Fig. 1. Acid-base equilibrium of harmalol, reported by Sarkar and Bhadraet, in the pH range 3–12. From Figure 1 in S. Sarkar and K. Bhadra, *Journal of Photochemistry and Photobiology B: Biology*, 2014, 130, 272–280 [1].

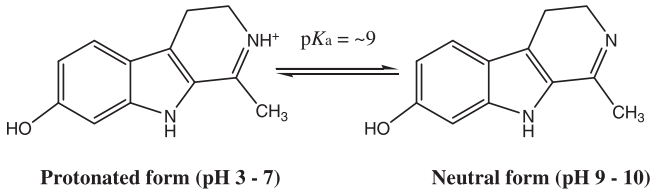

Fig. 2. Acid-base equilibrium of harmalol present in aqueous solution in the pH range 3–11, based on our results.

Under extreme alkaline conditions, Balon-Almeida et al. [5] described an additional equilibrium between the mono-anionic and the di-anionic species (forms c and d, respectively), with a pK_{a3} value of 16.0.

In addition, there is another point that should also be highlighted. Authors used a value of 42,280 M^{-1} cm^{-1} for the absorption coefficient (ε), at 371 nm, to calculate the concentration of the alkaloid solutions. Although they do not indicate to which acid-base species of harmalol this value corresponds, our experiments indicate that all the acid-base species of this alkaloid show ε values lower than 20,000 M^{-1} cm^{-1} (Fig. 3d). In particular, for the acidic species (i.e., form a) the corresponding ε, at 371 nm, is 15,904 M^{-1} cm^{-1}.

Therefore, the pK_a values and also the chemical structure for harmalol, as well as the ε values reported by Sarkar and Bhadra are in strong contradiction with all previous measurements [2–4,7] and our own present results. In the experimental section of their article, the authors do not describe how the pH was measured. At this point, we believe that the pH was not measured in an appropriate way. Furthermore, an incorrect assignment of the chemical structure for each acid-base species, as well as the use of a wrong ε values might have serious consequences in the conclusions reached in terms of the interaction with different biomolecules such as DNA.

Finally, authors stated that "the interaction of β-carboline alkaloids with DNA have been studied earlier but no detail work has been reported on the interaction of these various pH dependent forms with nucleic acid." However, there are several studies regarding the pH effect on the interaction between βCs and DNA published, [8–10] but these results are not taken into account by Sarkar and Bhadra.

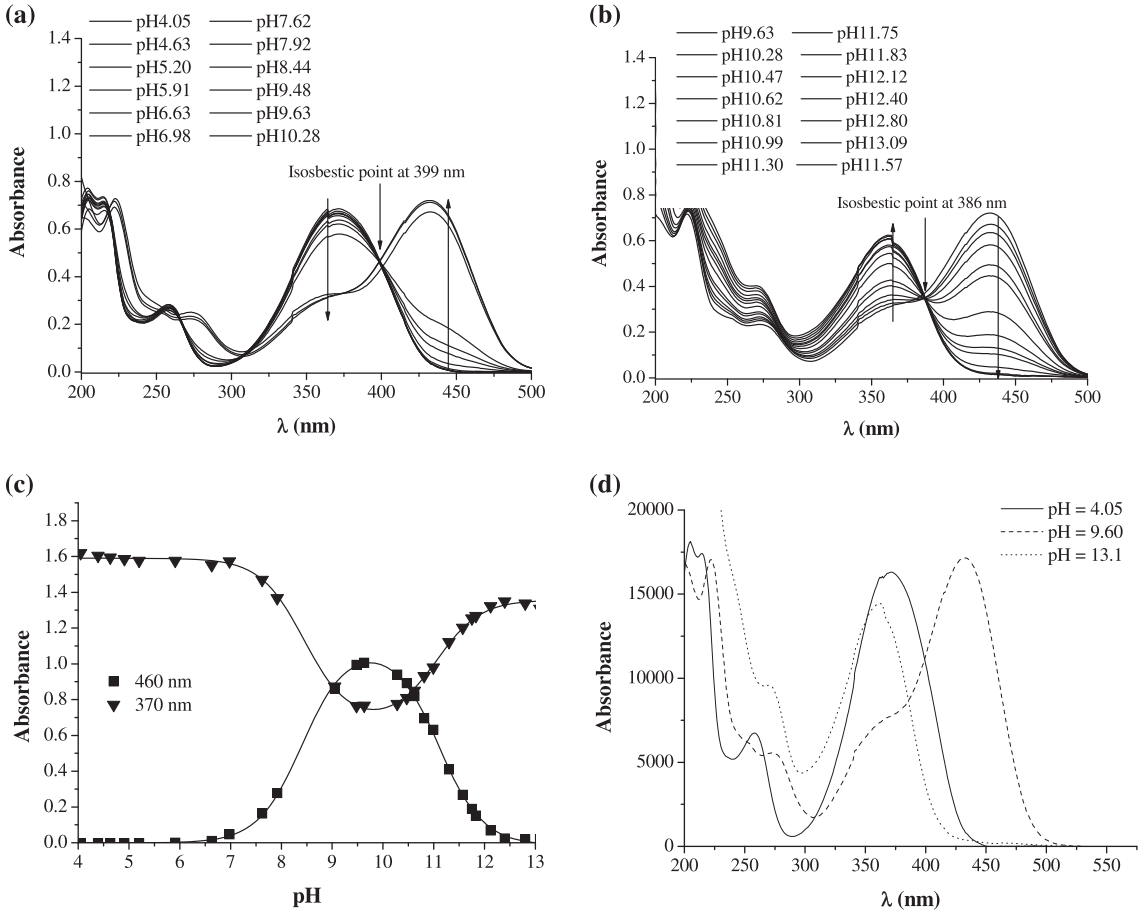

Fig. 3. (a) Absorption spectra of harmalol at different pH values ranged between 2 and 10. (b) Absorption spectra of harmalol at different pH values ranged between 10 and 13. All the spectra were recorded on quartz cuvettes with optical path length of 4 mm. (c) Spectrophotometric titration curves, at two different wavelengths, of harmalol (100 μM, optical path length of 10 mm; HCl and NaOH as titration agents). (d) UV–vis absorption spectra of each acid-base species of harmalol present in the whole pH-range investigated.

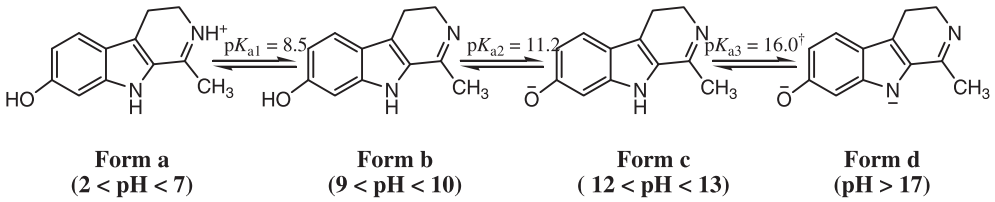

Fig. 4. Acid-base equilibria of harmalol, in its ground state, in aqueous solution under different pH conditions. † Value obtained from Ref. [5].

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