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COMMENTS

Comment on "Solvent Effect on the Electronic Spectra of Azine Dyes under Alkaline Condition"

Andrew Mills,*,[†] David Hazafy,[†] John A. Parkinson,[†] Tell Tuttle,[†] and Michael G. Hutchings[‡]

WestCHEM Department of Pure & Applied Chemistry, University of Strathclyde, Glasgow G1 1XL, U.K., and DyStar UK Ltd., Akcros Site, Lankro Way, Eccles, Manchester M30 0BH, U.K.

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Pal et al.[1] recently proposed that methylene blue (MB) is readily (within 1 h) converted to a nonionic, lipophilic, *N*-hydroxy adduct of MB, referred to as "MB−OH", when exposed to mild (0.1 mM) alkali (NaOH). MB−OH is readily extracted from the alkaline aqueous solution into toluene to form a red (λ_{max} = 526 nm) solution and is solvatochromic, but converted to a blue species, presumed to be MB, when spotted onto a silica-gel-coated TLC plate. The mass spectrum of MB−OH gave a molecular ion, *m/z* 301, appearing to confirm the formation of MB−OH. The structures of MB, MB−OH, and other species relevant to this work are given in Table 1.

The continuing importance of MB in various applications, most notably as a potential new treatment for Alzheimer's disease,[2] leads us to submit this Comment on the results of Pal et al.[1] All our attempts to reproduce the observations of Pal et al.[1] have failed. On following their simple experimental procedure we have found no toluene-soluble, red species was formed upon treating MB with 0.1 mM alkali, even after 24 h. Our observation of no reaction is not surprising given others have noted MB is "indefinitely stable" in aqueous solution at pH 9.5,[3] and that mixing MB with 0.1 mM alkali and shaking with a water-immiscible solvent (usually dichloromethane) is a published method for purifying MB of the demethylated thiazines, such as azure B (AB; usually the most prevalent species), which are common impurities in some commercial samples of MB.[4,5] This purification procedure is effective because the latter thiazines are readily deprotonated by alkali to their neutral, lipophilic orange or red forms, the structure of which for AB (i.e., dAB) is illustrated in Table 1.[5,6] Thus, it would seem that Pal et al.[1] have made an error in reporting the concentration of NaOH used in the production of MB−OH.

Encouragingly, it is possible to generate a species with near identical absorption spectra to those reported by Pal et al. for MB−OH[1] using the same method, but with 0.1 M, instead of 0.1 mM, NaOH aqueous phase solution shaken with toluene. We shall refer to the species generated using the higher alkali concentration and extracted into toluene as MB-red. If Pal et al.[1] used 0.1 M NaOH, rather than 0.1 mM, then we should expect MB-red to be the hydroxy adduct MB−OH. Promisingly, MB-red shows the same solvatochromic properties as reported by Pal et al.[1] for MB−OH and turns blue when spotted onto a silica-gel-coated TLC plate. These observations would seem to indicate that our red-MB and MB−OH are one and the same.

It is with concern, therefore, to note that subsequent experiments reveal MB-red to be largely methylene violet (Bernthsen), MVB (Table 1). The latter is available commercially and has spectral and physical properties identical to MB-red. For example, MVB is lipophilic and has the same solvatochromic UV/visible absorption characteristics as MB-red (which in turn are near-identical to the species identified as MB−OH). MVB and MB-red also fluoresce with a maximum of emission at 596 nm in toluene (λ_{excit} = 520 nm), in agreement with the literature.[7] As noted above, when MB-red (like MB−OH) in toluene solution is spotted onto a silica TLC plate, the spot turns blue, but on development this spot separates into two, neither of which is MB. The most striking spot in terms of depth of color has the same retention time, R_f, as commercial MVB, while the other, which is much weaker in color, has the R_f value of AB.

The NMR spectra recorded for MB (in D_2O; 500.13 MHz), MB-red, and MVB (in CCl_4; 600.13 MHz) reveals MB-red to be identical to MVB. (It is not clear why Pal et al.[1] report MB−OH to be too unstable to record NMR spectra, yet stable enough to record visible spectra at room temperature in solvents such as $CHCl_3$ and CCl_4.[1]) Consistent with the above, the mass spectrum of MB-red in toluene reveals the predominant presence of MVB (molecular ion peak at *m/z* 256) with some AB (*m/z* peak at 270). The latter observations are in contrast to the work of Pal et al.,[1] who observed a molecular ion peak at *m/z* 301,[1] although in the mass spectrum of MB−OH shown in their Supporting Information, there are also substantial peaks at *m/z* 256 (not 257 as Pal et al.[1] state), 270, 320, and 342,[1] and it is difficult to rationalize the latter two fragments in terms of MB−OH.

A key justification, used by Pal et al.[1] and earlier by Plater,[8] for *N*-hydroxylation leading to the formation of MB−OH is the proposal that the central heterocyclic nitrogen in MB is electron deficient. However, this is not supported by the literature cited by Plater,[8] who also notes in his ref 15 that MB is hydrolyzed to MVB. Contrary to the suggestion of an electropositive central N in MB, our analysis of DFT calculations on MB applying both the NBO and electrostatic potential (ESP) methods to determine the charge distribution of MB reveals that the central heterocyclic N atom in fact has an overall partial *negative* charge (NBO: −0.38e, ESP: −0.79e; see Figure 1 in the Supporting Information). Such a situation vitiates the basis proposed[1,8] for nucleophile addition at the heterocyclic N atom and renders formation of the *N*-hydroxy adduct (MB−OH, Table 1) unlikely. In addition, TD-DFT calculations reveal that MB−OH would have an absorption maximum in the near UV at 351 nm, i.e., a species with the structure shown for MB−OH would be colorless,

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* Corresponding author. E-mail: a.mills@strath.ac.uk.

[†] WestCHEM Department of Pure & Applied Chemistry, University of Strathclyde.

[‡] DyStar UK Ltd.

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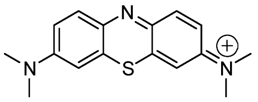
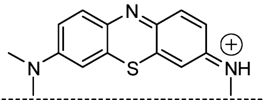
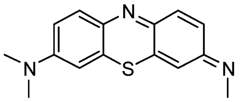
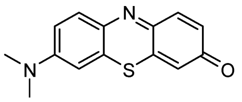
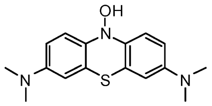
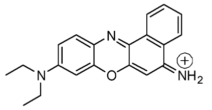
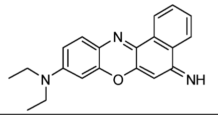
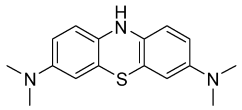
TABLE 1: Summary of Structural, Spectral, and Physical Characteristics of MB and Other Relevant Dyes

Cationic dye structure	Abv./name Mol. weight	$\lambda_{\mathrm{max}}(\mathrm{H_2O})$ /nm	λ_{max}(toluene) (λ_{max}(calc.**))/nm
	MB Methylene blue 284.4	665	n/a
	AB Azure B 270.37	645	n/a
	dAB deprot. AB 269.36	n/a	503 (521)
	MVB Methylene violet 256.32	610	520 (500)
	MB-OH 301.41	662*	526* (351)
	NB Nile blue 318.39	635	n/a
	dNB deprot. Nile blue 317.38	n/a	500 (492)
	LMB *leuco*- Methylene blue 285.41	256***	n/a

* Reference 1. ** Calculated using TD-DFT. *** Reference 9.

as expected. (MB−OH is simply the *N*−OH analogue of the well-known colorless reduced form of MB, leuco methylene blue (LMB); see Table 1.[9]) Interestingly, G. N. Lewis et al. in related chemistry more than 60 years ago had speculated on the formation of MB−OH by the addition of water to MB and noted that if formed it would be colorless.[10]

In conclusion, our findings indicate that the red-MB species observed by Pal and co-workers,[1] and claimed to be the *N*-hydroxy adduct MB−OH, is in fact MVB, possibly containing some AB, both of which are formed by hydrolysis. The observation here and by others that AB is also rendered lipophilic by deprotonation brings into question the reported[1] formation of a colored hydroxy adduct of nile blue (NB; an oxazine dye; Table 1), when deprotonation of the amine group (just as in AB) to form dNB appears much more likely (see Table 1).

Supporting Information Available: Description of computational methods and a figure illustrating calculated charge distribution for MB using the NBO and ESP charge models. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

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