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Molecular Switches Hot Paper

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Light-Switchable Buffers

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Abstract: *A visible light-switchable buffer system based on a merocyanine photoacid is presented. Para-substitution of the indolium side with a methoxy group affords a compound suitable for making hydrolytically stable aqueous buffers whose pH can be tuned between 7 and 4 using 500 nm light.*

Since the introduction of the pH scale and the word "Puffer" (in German) into the chemical lexicon by Søren P. L. Sørensen[1] in 1909, buffer solutions constitute an essential tool in the study of aqueous (bio)chemical systems.[2] By definition, buffers can better resist pH changes when their actual pH equals the pK_a of the acid-base pair they are composed of—i.e., their buffer capacity is maximized.[3] Herein, we introduce the possibility of making buffer solutions whose pH can be set at different constant values according to a light-triggered acid-base pair's pK_a shift.

Protonated merocyanines (MCHs) are spiropyran compounds featuring negative photochromism.[4] They are commonly referred to as "metastable-state photoacids"[5]—i.e., chemical species whose proton photo-dissociation is persistent enough to be coupled efficiently and reversibly with proton transfer reactions in solution.[6] For this reason, MCHs are attracting increasing attention across multiple research fields spanning molecular machines,[7] supramolecular chemistry,[8] nanotechnology[9] and materials science.[10] Recently, we studied a series of MCHs bearing alkyl-1-sulfonate bridges of variable length, providing insights on their thermodynamics and kinetics in water (Figure 1a) through cross-validation of ^{1}H NMR, UV/Vis, and pH measurements.[11] We showed that, under dark conditions, MCHs are organic weak acids displaying a ground state acidity constant close to neutrality (pK_a^{GS} = 6–7), whereas,

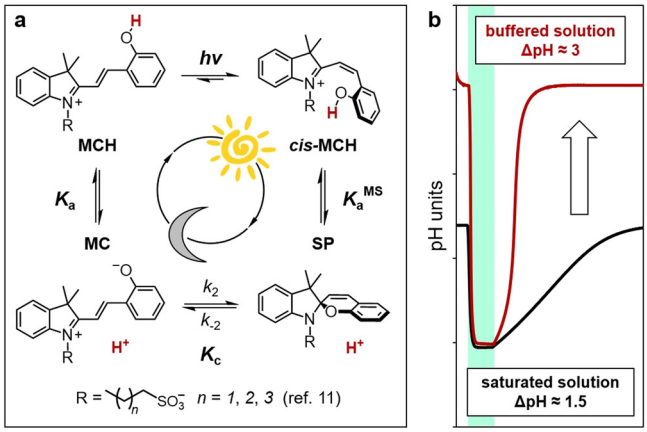

Figure 1. a) Four-state model describing the operation of MCHs in water. Under dark conditions, dissociation (K_a) of MCH is followed by isomerization (K_c) of MC to the corresponding SP form—i.e., $K_a^{GS} = K_a(1+K_c)$. Under steady light irradiation, photoproduct *cis*-MCH undergoes ring-closing with concomitant release of a proton (K_a^{MS}). Liao's photoacid and all compounds discussed here bear a propyl-1-sulfonate group (n = 2). b) Effect of buffering on 500 nm light-triggered pH jumps reported in this work.

under steady light irradiation, they became more acidic by ca. 4 pK units (pK_a^{MS} = 2–3).[11] The extent of proton release (ΔpH) that a given MCH can achieve in solution results from a delicate interplay between its photoacidity (Π = pK_a^{GS} − pK_a^{MS}), quantum yield (Φ) and solubility, and usually lays within 1.5 pH units. Here, we expand our experimental and theoretical approaches to methoxy-substituted MCHs, dissecting the possibility of achieving reversible pH changes as high as Π, by using buffered solutions (Figure 1b)—i.e., MCHs' aqueous solutions whose dark equilibrium composition is not quantitatively shifted towards the undissociated form, MCH. In particular, we will show that the pH in the dark can be set at different constant values according to the Henderson-Hasselbalch equation and that ΔpH can be controlled—from 1 to 3 pH units—by carefully tuning the intensity of the light source.

Working with MCHs in aqueous environments can be problematic due to their tendency to hydrolyze irreversibly.[12] MCHs hydrolyze with a mechanism similar to that of Schiff bases (Figure 2a).[11] We reasoned that *ortho*- or *para*-substitution of either the indolium or the chromene moiety with electron-donating groups might increase MCHs' hydrolytic stability by disfavoring water nucleophilic attack. Therefore, we decided to quest for favorable mesomeric effects by screening four different MCHs bearing a methoxy group directly conjugated to the ene-iminium core. Compounds **1**–**4** were synthesized according to standard literature procedures

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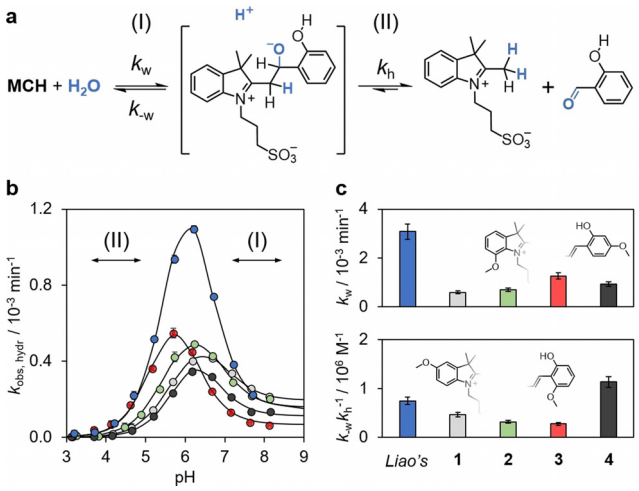

Figure 2. a) Mechanism of hydrolysis of Liao's photoacid below pH 9 (see ref. [11]). b) Profiles of apparent first-order rate constant of hydrolysis of Liao's photoacid (blue), compound **1** (grey), **2** (green), **3** (red), and **4** (black) as a function of the pH. Solid black lines represent the best fits to Equation (S1). c) Extrapolated kinetic parameters for nucleophilic addition of water (top) and decomposition of the tetrahedral intermediate (bottom). Experimental conditions: [**1**–**4**] = 25 ± 2 μM, [phosphate buffers] = 20 mM, T = 25 °C.

and characterized by common techniques including X-ray crystallography[13] (see SI for more details). We studied their hydrolytic stability as a function of the pH in water by UV/Vis spectroscopy, monitoring the decay of the corresponding MC(H) form at 25 °C (Figure S1). The resulting hydrolysis profiles are reported in Figure 2b together with that (highlighted in blue) previously obtained for Liao's photoacid, which we take as reference.

In all cases, Equation (S1) fits the obtained bell-shaped profiles with good confidence, confirming that the rate-determining step of MCHs' hydrolysis changes with pH from decomposition of a tetrahedral intermediate (II) to nucleophilic addition of water (I) (see SI for more details). Inspection of the obtained kinetic parameters (Figure 2c) revealed that water nucleophilic addition proceeds most slowly in the case of **1** (grey) and that the intermediate reacts favorably—either backward or forward—only in the case of **4** (black). These preliminary findings indicate that *para*-substituted MCHs are better able to resist hydrolysis over the entire pH window tested. Thus, we decided to continue our investigations with compounds **1**[14] and **4**[12] for practical convenience.

We moved on studying the MC ⇌ SP isomerization, monitoring the decay of the corresponding MC form at 25 °C. Kinetic studies were carried out at pH 9.5, where competing hydrolytic processes are limited, and the dark equilibrium is reduced to the MC and the SP forms only. In the case of **1**, we determined an isomerization constant $K_c = 1.46 \pm 0.07$ (Figure S2). This value indicates that **1** has less tendency to equilibrate towards SP in the dark as compared to Liao's photoacid, whose K_c lays around 9 ± 1.[11] However, the rate constant of back-isomerization (k_{-2}) was found to be similar ($0.0044\ s^{-1}$ vs. $0.0045\ s^{-1}$), suggesting that **1** may possess comparable photochemical performance (see below). As for compound **4**, the MC ⇌ SP isomerization was so fast that we were not able to determine any thermodynamic and kinetic constant under the same conditions. An alternative way to assess k_{-2} is to follow thermal relaxation kinetics after irradiation. The observed rate constant of relaxation in the dark matches k_{-2} in the pH range between pK_a^{MS} and pK_a—i.e., at around pH 5 (see SI for more details).[11] Therefore, we set up kinetic analyses at pH 5.2, monitoring the recovery of the corresponding MCH form at 25 °C after light irradiation (Figure S3). We found that **1** undergoes quantitative photoisomerization (> 99 %) within a few milliseconds, whereas **4** isomerizes to a dramatically lesser extent (ca. 13 %). The observed rate constants of relaxation in the dark were found to be $0.0043\ s^{-1}$ for **1**—in good agreement with the k_{-2} value estimated at pH 9.5—and $4.5\ s^{-1}$ for **4**. Regrettably, this means that the half-life of proton photo-dissociation of compound **4** is nearly three orders of magnitude shorter than that of **1** (milliseconds vs. seconds). It follows that **4** would operate similarly to **1** only by using more intense light sources, which however we do not have. For this reason, we continued our studies focusing on compound **1**.

Acid dissociation constants of **1** in the dark and under steady light irradiation were determined by UV/Vis spectroscopy, probing the dark equilibrium composition or the photostationary state at increasing pH values. According to the MC ⇌ SP isomerization studies described above, all spectra in the dark were recorded after an equilibration time of at least 15 minutes at 25 °C. In fact, only by doing so the obtained signals refer to an equilibrium situation. Under dark conditions, UV/Vis pH titrations of **1** (Figure 3a) gave an apparent pK_a^{GS} value of 6.95 ± 0.03. This result was confirmed by ^{1}H-NMR analyses, which revealed that **1** features a $pK_a = 7.4 \pm 0.1$ along with a $K_c = 1.4 \pm 0.1$, resulting in a $pK_a^{GS} = 7.0 \pm 0.1$ (Figure S5). On the other hand, photochemical studies under steady light irradiation showed that **1** displays slightly different photostationary states depending on the wavelength of irradiation, featuring an apparent pK_a^{MS} value decreasing from 3.43 ± 0.04 at 425 nm (Figure S6) to 3.31 ± 0.04 at 500 nm (Figure 3b). These studies show that the photoacidity of compound **1** ($\Pi_{500} = 3.6 \pm 0.1$) is similar to that of Liao's photoacid ($\Pi_{425} = 3.7 \pm 0.1$[11]) but more shifted towards the neutral region by ca. 1 pK unit, and that **1** can be easily activated by exposure to visible light of longer wavelengths.

We then examined the reversible proton release of compound **1** in water. pH readings at equilibrium in the dark were fully consistent with pK_a^{GS} and the total concentration of **1** in solution. Simultaneous data fitting of our four-state model to three independent variable-power pH-jump experiments carried out using either 425 or 500 nm light sources (Figures S8) revealed that the quantum yield does not change significantly by shifting the wavelength of irradiation, yielding an average value of $\Phi_{425} \approx \Phi_{500} = 0.40 \pm 0.03$. This value is close to the one obtained for Liao's photoacid by Coudret and co-workers[15] ($\Phi_{436} = 0.38 \pm 0.03$) and confirms the hypothesis that **1** does display comparable photochemical performance.

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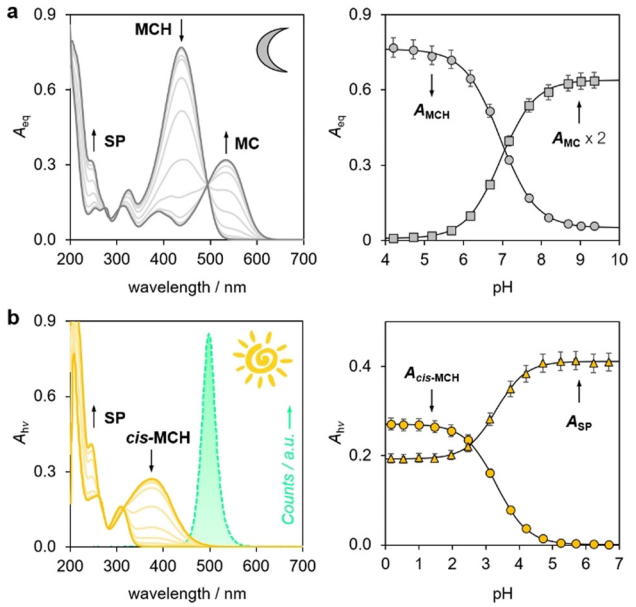

Figure 3. UV/Vis spectra of **1** in the dark (a) and under steady light irradiation (b) as a function of the pH. The obtained absorbance (*A*) profiles of MCH (437 nm), MC (534 nm), *cis*-MCH (375 nm), and SP (225 nm) are reported on the right. Solid black lines represent the best fit to Boltzmann's sigmoidal equation. Experimental conditions: [**1**] = 24 ± 2 μM, [phosphate buffers] = 20 mM, T = 25 °C. In the case of (b), spectra were acquired using a 500 nm LED-light source (100 mW); its emission spectrum is highlighted using the corresponding RGB color code.

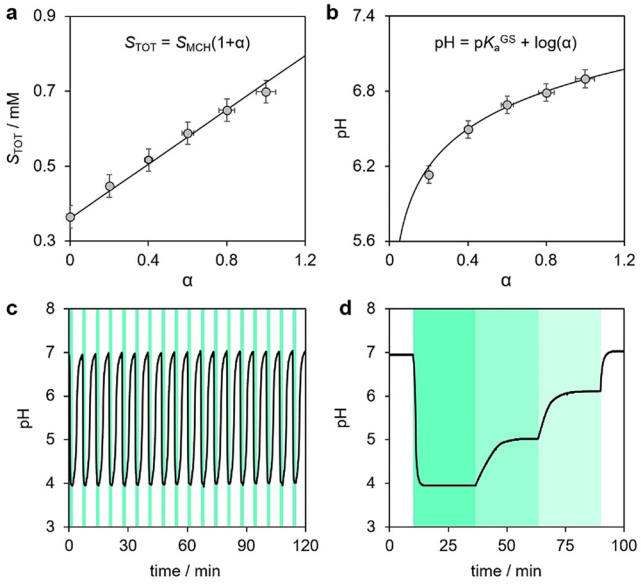

Figure 4. a) Apparent solubility of compound **1** in the dark as a function of the equivalents of NaOH (α) added to the system. b) The pH of the resulting buffer solutions at equilibrium in the dark as a function of α; solid black lines represent the best fit to Equation (1) and (2), respectively. c) Reversible jumps of a solution of **1** at $pH = pK_a^{GS}$ ($\alpha = 1$). d) pH tuning by modulation of the light source power. Experimental conditions: T = 25 °C, 500 nm LED-light source: (c) 195 mW and (d) 40.0, 8.2 and 7.0 mW.

We moved on assessing the possibility of controlling reversible pH changes in buffered solutions. First, we wanted to see whether and how the solubility of **1** changes as a function of the equivalents of base added to the system—indicated hereafter with the Greek symbol α. Solubility experiments were carried out stirring an excess amount of crystals into aqueous KCl containing HCl (1 mM) or increasing sub-stoichiometric amounts of NaOH. These experiments were performed at a constant temperature of 25 °C until stabilization of the pH (Figure S9). Compared to Liao's photoacid, we observed that the solubility of the undissociated form **1**-MCH (S_{MCH}) is higher, but still remains in the sub-millimolar regime (0.37 mM vs. 0.19 mM). Interestingly, however, we found that the total amount of **1** (S_{tot}) that can be dissolved in aqueous KCl increases linearly with α (Figure 4a) according to the following equation:

$$S_{tot} = S_{MCH}(1+\alpha) \quad (1)$$

We ascribed the occurrence of such a linear regime to a situation where the dissociated forms **1**-MC and **1**-SP are effectively more soluble than **1**-MCH, resulting in a Le Châtelier shift that maintains S_{MCH} independent from α.

The pH values of the resulting buffer solutions at equilibrium were found to be in good accordance (Figure 4b) with those predicted by the Henderson-Hasselbalch approximation,[16] which, after deriving the expression for the concentration of both the dissociated and undissociated forms from Equation (1), can be rewritten as:

$$pH = pK_a^{GS} + \log(\alpha) \quad (2)$$

All in all, these observations show that the pH in the dark (and so the hydrolytic stability, see Figure 2b) can be controlled and raised at will without diminishing the concentration of the photoactive form (**1**-MCH). Independent pH-jump experiments showed that ΔpH increases significantly with α, passing from 1.5 to 3.0 pH units from $\alpha = 0$ to $\alpha = 1$. Importantly, the obtained profiles of proton release/uptake were fully in line with model predictions (Figure S11). In the case of the buffer solution at $pH = pK_a^{GS}$ we were able to trigger fully reversible jumps of 3 pH units for 2 consecutive hours without significant loss (Figure 4c). In a separate experiment, we also demonstrated that the amplitude of the pH jump can be controlled at will by modulating the intensity of the light source (Figure 4d).

The results presented here show that our four-state model can be extended to describe MCHs' proton release/uptake in buffered systems. We believe our findings hold promise for implementing MCHs in the remote control of any aqueous acid-sensitive (bio)chemical system with visible light.

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Conflict of Interest

The authors declare no conflict of interest.

Keywords: buffers · molecular switches · photoacidity · protonated merocyanines · water

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