Compartmental Fluorescent pH Indicators with nearly Complete Predictability of Indicator Parameters; Molecular Engineering of pH Sensors

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The fluorescent pH indicator parameters for compounds (1a) and (1b) are predictable within experimental error from the appropriate data for the constituent units 2- or 4-methyl benzoic acid and the fluorophore (1c), except for the maximum fluorescence quantum yield which is predictable to within an order of magnitude.

We recently showed how the principle of photoinduced electron transfer[1] can be employed to design fluorescent pH indicators whose favourable features arose from their compartmental nature.[2] We now (a) generalize this approach

beyond aromatic hydrocarbon–amine systems, (b) show that the optical properties of these indicators can be tuned by variation of substituents over a moderate wavelength range while pH indicator action is maintained, and (c), most importantly, demonstrate that the compartmental nature can be exploited to yield systems with nearly complete predictability of all pH indicator parameters. This makes possible, for the

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Table 1. Properties of compounds (1).[a]

R¹	Absorption[b,c] $\lambda_{max.}$/nm (10^4 $\varepsilon_{max.}$/dm³ mol⁻¹ cm⁻¹)	Emission[c,j] $\lambda_{max.}$/nm[b]		ϕ_{Fmax}	ϕ_{Fmin}[e]	Electrochemical $-E_{red(D)}$/V[f]	ΔG_{ET}/eV[g]		pK_a*	pK_a
OMe	354 (2.2)	470	(1a)	0.037	0.002	2.49	−0.35	(1a)	4.2	4.6
			(1b)	0.12	0.004			(1b)	3.9	3.7
			(1c)	0.20	0.19					
H	359 (2.2)	475	(1a)	0.13	0.003	2.34	−0.20	(1a)	4.2	4.4
			(1b)	0.20	0.004			(1b)	3.9	3.7
			(1c)	0.19	0.18					
Ph	361[h] (2.4)	485	(1a)	0.25	0.008	2.13	+0.01	(1a)	4.6	4.4
			(1b)	h	h			(1b)	h	h
			(1c)	h	h					
CN	398 (2.9)	510	(1a)	0.30	0.20	1.2	+0.9	(1a)	4.2	4.5
			(1b)	0.31	0.29			(1b)	i	3.8
			(1c)	0.35	0.35					

[a] Aerated aqueous solutions with 20% (v/v) methanol to aid solubility of (1a) and (1b) (10^{-5} M). pH range 2.5—12, 29 °C. μ = 0.1 M (NaCl). Compounds (1c) and (1a, R^1 = H) are known (ref. 6) and the others were prepared by analogous procedures and had the expected spectral properties. [b] All values are pH independent (when observable) and are essentially identical for each set of (1a—c) [c] In these experiments where (1c) was also examined, adequate solubility required the use of 50% (v/v) methanol: the $S_0 \rightarrow S_1$ (and $S_1 \rightarrow S_0$) spectra of each set (1a—c) are superimposable. [d] Excited state acidity constants pK_a* obtained from ϕ_F–pH profiles and the relation $\log [(\phi_{Fmax.} - \phi_F)/(\phi_F - \phi_{Fmin.})] = -pH + pK_a*$. Ground state acidity constants pK_a obtained from solubility (S)–pH profiles and the relation $\log [(S/S_{min.})-1] = pH - pK_a$. 4- and 2-Methyl benzoic acid have pK_a 4.5 and 4.1 respectively (potentiometric titration) in the medium specified in footnote a. [e] The methyl ester of (1a, R^1 = H) (ref. 6) has a pH-independent ϕ_F value of 0.003. [f] Values for (1c) from F. Pragst and F. G. Weber, J. Prakt. Chem., 1976, **318**, 51. The value for (1c, R^1 = CN) was interpolated from a Hammett type correlation of their larger data set. [g] For photoinduced electron transfer from the pyrazoline moiety to the benzoic acid moiety of the acidic form of (1a) or (1b) $\Delta G_{ET} = -E_s + E_{ox(D)} - E_{red(A)} - e^2/\varepsilon r$ (D. Rehm and A. Weller, Isr. J. Chem., 1970, **8**, 259) and $E_s = E_{ox(D)} - E_{red(D)}$. $e^2/\varepsilon r$ is taken as 0.1 eV (Z. R. Grabowski and J. Dobkowski, Pure Appl. Chem., 1983, **55**, 245). L. Meites and P. Zuman ('Electrochemical Data,' Part 1, vol. 1A, Wiley, New York, 1974) give $E_{red(A)}$ for PhCO₂R (R = Me, H) = −2.24 V. The differing conditions of solvent and redox reversibility will only allow an estimate of the ΔG_{ET} value for our experiments. The corresponding ΔG_{ET} values for the basic form of (1a) or (1b) are expected to be substantially more positive. This is the rationale behind the pH switching ('on' in base and 'off' in acid) of (1a) and (1b). [h] Data unavailable owing to insufficient solubility. [i] Insufficient difference between ϕ_{Fmax} and ϕ_{Fmin} to permit evaluation of pK_a*. [j] ϕ_F Values were obtained by comparison of the areas of corrected spectra with that of 9,10-diphenylanthracene (ref. 7 gives ϕ_F = 0.95 in deaerated ethanol). These ϕ_F determinations were facilitated by the independence of spectral shapes with respect to R^2 and pH. The reported λ_{max} values are from uncorrected spectra in view of their smaller uncertainty.
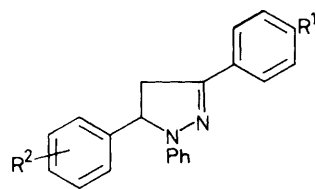
(1)

a; R^2 = 4-CO₂H/CO₂⁻
b; R^2 = 2-CO₂H/CO₂⁻
c; R^2 = H

first time, the design of fluorescent pH indicators satisfying the particular optical and pH requirements of a given application.[3] Previously, the development of fluorescent pH indicators has been empirical.[4]

The indicator series (1a) and (1b) was designed by joining the acid/base unit 2- or 4-methylbenzoic acid with the fluorophore, 1,5-diphenyl-3-aryl-4,5-dihydro-pyrazole (1c), which are well known optical brighteners.[5,6] The results in Table 1 show that (a) ΔG_{ET} is a good predictor of the occurrence or absence of practical pH indicator action. The pH switching of fluorescence is good (ϕ_{Fmin}/ϕ_{Fmax} <0.06) in all cases except when ΔG_{ET} is substantially positive in which

case the pH switching is unremarkable (1a, R^1 = CN) or virtually nonexistent (1b, R^1 = CN). (b) All the electronic absorption and emission spectral parameters for (1a) and (1b) except the fluorescence quantum yield (ϕ_F) are pH independent and are identical, within experimental error, to the corresponding parameters for (1c). (c) The pH dependence of ϕ_F is predicted well by the Henderson–Hasselbalch type equation $\log [(\phi_{Fmax} - \phi_F)/(\phi_F - \phi_{Fmin})] = -pH + pK_a*$. (d) ϕ_{Fmax} Values for (1a) and (1b) are lower than, but within a factor of six of, the pH independent ϕ_F values for (1c). (e) pK_a* = pK_a[8] and furthermore is identical, within experimental error, to the pK_a of the corresponding methyl benzoic acid. Thus all the pH indicator parameters, both optical and acid/base, of (1a) and (1b) can be quantitatively predicted with good accuracy (except for the somewhat poorer performance of ϕ_{Fmax}) from the easily available optical, electrochemical, and acid/base parameters of the simple constituent molecules 2- or 4-methylbenzoic acid and (1c).

Our previous system, the dialkylamino methyl anthracenes,[2] showed some of these aspects of predictability but the observed pK_a* (= pK_a) values differed by ca. three pH units from the pK_a values of the corresponding acid/base constituents, the simple trialkylamines. Such a large deviation presumably arose from steric hindrance by the 9-anthryl moiety to the solvation of the protonated amine module. The good predictability of the fluorescent pH indicator properties

of (1a) and (1b) must arise since the core of the acid/base unit is rigidly held remote[9] [especially in (1a)] from the fluorophore while electron transfer between the two π electron systems across a single saturated carbon atom can take place readily.

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