Solvent dependence of pyranine fluorescence and UV-visible absorption spectra

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ABSTRACT: Fluorescence and UV–visible absorption spectra of HPTS (8-hydroxy-1,3,6-pyrenetrisulphonic acid trisodium salt, pyranine) were measured in a variety of solvents. Fluorescence maxima (in kcal mol^{-1}) can be correlated with the Kosower Z parameter ($r = 0.901$), the Dimroth–Reichardt $E_T(30)$ parameter ($r = 0.900$) and the Winstein Y parameter ($r = 0.916$) using one-parameter fits. Good correlations ($r = 0.98$) were obtained for HPTS fluorescence in ethanol–water mixtures using the Y, Y_{OTs} and Z parameters. Fluorescence maxima of HPTS in aqueous sulphuric acid solutions gave an excellent correlation with Y_{OTs} ($r = 0.991$). Multi-parameter correlations, indicating the significance of specific solvent interactions, were also studied. In addition, fluorescence maxima correlate well with maximum/minimum ratios obtained from UV–visible absorption experiments. Results can be applied in the use of HPTS as a molecular probe of solvent environments and for extension of the Y_{OTs} scale in acidic solutions. HPTS is a unique molecular probe, not only because of its photoacidic properties and its widespread use as a pH-sensitive biosensor, but also because of its relative stability in acidic environments. © 1998 John Wiley & Sons, Ltd.

KEYWORDS: pyranine; photoacid; solvent polarity parameters; solvatochromism

INTRODUCTION

The fluorescence of HPTS and its application have been studied for nearly half a century.[1–14] Förster,[1,2] Weller[3,4] and co-workers first measured the pH-dependent fluorescence of HPTS, due to its ROH* and RO$^-$* forms, and established indirect methods of excited state proton transfer rate determination. Excited state proton transfer from HPTS has since been studied directly.[5,6] HPTS fluorescence has been used in scientific, medicinal and commercial applications. For example, HPTS has been used to study the process of carbon monoxide binding to haemoglobin;[7] as a pH probe of liposome interiors and surfaces;[8] and in sensors of pH,[9,10] carbon dioxide[11] and ammonia.[12]

In addition to the pH dependence of HPTS fluorescence, the maximum of the ROH* fluorescence is solvent-dependent. However, there have been only a few studies of this solvent dependence. Kondo *et al.*[13] used the solvent dependence in order to understand reversed micelles, and Wolfbeis[9] noted it (using a crown ether) in applications to pH measurements. Yuqing and

Longdi[14] have proposed that HPTS may be used as a polarity probe owing to its solvent dependence.

Because of the widespread use of HPTS, we felt that a more complete study of its solvent dependence was necessary. Therefore in this study we report preliminary results on the correlation of HPTS fluorescence and UV–visible absorption spectra with the Kosower Z[15] (reference numbers indicate the source of values used), Dimroth–Reichardt $E_T(30)$,[16,17] Winstein Y,[15] Y_{OTs}[18] and Py[19] solvent polarity parameters. Multi-parameter correlations with the Swain A and B scale,[15] the Kamlet–Taft scale[16,20] and the Krygowski–Fawcett ($E_T + DN$)[15] scale are also presented. Results for 30 solvents are given. The effect of added salt on HPTS fluorescence is discussed in a separate publication.[21]

Macroscopic solvent parameters such as the dielectric constant or the index of refraction have limited use in understanding solvent-dependent properties of a particular solute or reaction, since they do not measure specific solute–solvent interactions such as hydrogen bonding or electron pair donor–acceptor interactions.[15,22,23] Parameters such as the Kosower Z value or the Dimroth–Reichardt $E_T(30)$ value are frequently used instead.[15,22,23] These parameters measure both non-specific and specific solvent–solute interactions of a particular probe molecule and thereby permit one to discern which solvent–solute properties are of primary importance for the process of interest.

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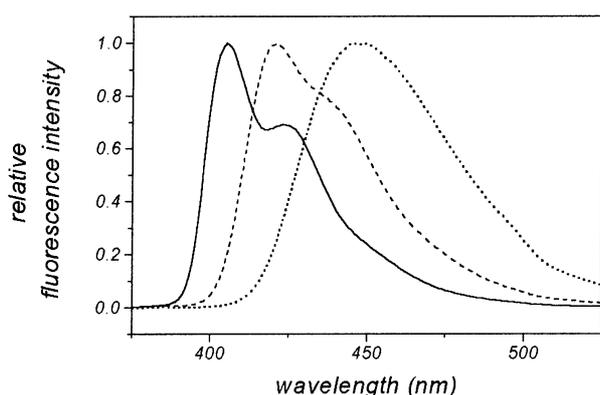
Figure 1. Fluorescence spectra of HPTS in: acetonitrile (with 1% methanol by volume added to improve solubility), full line; ethanol, broken line; and 30% HCl solution (by weight), dotted line

RESULTS AND DISCUSSION

Fluorescence spectra

One-parameter correlations. Fluorescence spectra of HPTS in acetonitrile (containing 1% methanol by volume to improve solubility), ethanol and 30 w/w% hydrochloric acid are shown in Fig. 1. The spectra arise from fluorescence of ROH* and typify the positive solvatochromism observed for the HPTS fluorescence. A more extensive list of fluorescence data is given in Tables 1 and 2.

The Kosower Z value scale is based on the intermolecular charge transfer transition of 1-ethyl-4-(methoxycarbonyl)pyridinium iodide. A graph of the energy of HPTS fluorescence maxima versus Z is given in Fig. 2(a). (HPTS has low solubility in non-polar solvents as well as in some polar aprotic solvents, so several solutions were prepared by mixing HPTS in a small amount of methanol and then adding the mixture to the solvent of interest. Z values and $E_T(30)$ values for the corresponding methanol–acetonitrile and methanol–acetone mixtures were calculated according to information given in Refs 15 and 17. The data set also includes several data points for which solvent parameters are unavailable.) The correlation coefficient using all possible points is 0.901. Owing to the size of the data set, uncertainties in data due to low solubility, and selection of appropriate values of polarity parameters for mixtures and for water, the correlation

Table 1. HPTS fluorescence and UV–visible absorption data

Solvent	Fluorescence maximum (kcal mol^{-1})	Absorption ratio (max. min.)	Large data set	Small data set	Y value data set
Acetic acid (1:200 MeOH:acetic acid)	66.4	1.45	×	×	
Acetic acid (glacial)	66.8	1.80	×		×
Acetone (1:50 MeOH:acetone by volume)	70.6	2.35	×	×	
Acetone (neat)	73.6	–	×		
Acetonitrile (1:100 MeOH:MeCN)	70.6	2.38	×		
Acetonitrile (1:150 MeOH:MeCN)	70.6	2.31	×		
Acetonitrile (neat)	71.3	2.46	×	×	
CF$_3$SO$_3$H	61.2	1.07	×	×	
DMF (*N,N*-dimethylformamide)	70.1	2.21	×	×	×
DMSO	69.6	2.32	×	×	
Ethanol	68.0	1.99	×	×	×
Ethylene glycol	67.3	1.81	×	×	
Formic acid	65.3	1.64	×		×
H$_2$SO$_4$ (98%)	62.1	1.08	×		
H$_3$PO$_4$ (85%)	63.4	1.53	×	×	
H$_3$PO$_4$ (88%)	63.4	1.40	×		
HCl (30%)	64.0	1.54	×	×	
HClO$_4$ (70%)	62.7	1.52	×		
Methanol	67.9	2.00	×	×	×
1-Propanol	67.9	1.92	×	×	
2-Propanol	68.7	–	×	×	×
Pyridine	74.2	–	×		
t-Butanol	68.5	–	×		×
2,2,3,3-Tetrafluoropropanol	66.6	1.56	×	×	
2,2,3,3-Tetrafluoropropanol (1:150 MeOH:TFP)	66.3	1.57	×	×	
2,2,3,3-Tetrafluoropropanol (1:300 MeOH:TFP)	66.6	1.56	×		
THF	75.2	–	×		
2,2,2-Trifluoroethanol	67.3	1.70	×		×
2,2,2-Trifluoroethanol (1:150 MeOH:TFE)	66.6	1.70	×	×	
Water (average of solutions containing 0.5–1.5 M acid, to detect ROH fluorescence)	64.5	1.62	×	×	×

Table 2. One-parameter correlations with fluorescence data

Parameter	Data set[a]	Correlation coefficient
$E_T(30)$	Large	0.900
	Small	0.938
	Y	0.863
	Protics	0.823
	Aprotics	0.888
Z	Large	0.901
	Small	0.946
	Y	0.805
	Protics	0.790
	Aprotics	0.880
Y	Large	0.916
	Small	0.982
	Protics	0.915
	Aprotics	[b]
Y_{OTs}	Large	0.885
	Small	0.926
	Y	0.932
	Protics	0.923
	Aprotics	[b]
Py	Protics	0.830
	Aprotics	0.961
DN	Protics	0.863
	Aprotics	0.165

[a] Data sets as indicated in Table 1.
[b] Insufficient data.

Figure 2. Correlations of HPTS fluorescence energy with (a) Kosower Z scale and (b) normalized Dimroth–Reichardt $E_T(30)$ scale

coefficient is dependent upon the choice of data. Correlation with a smaller data set (of 17 points, which excludes several solvents in which HPTS did not easily dissolve or that gave poorer correlations; see Tables 1 and 2) gives a correlation coefficient of 0.946.

Dimroth–Reichardt $E_T(30)$ values, which are based upon the probe molecule 2,6-diphenyl-4-(2,4,6-triphenyl-1-pyridinio) phenolate, are affected by solvent Lewis acidity and polarity.[15] A graph of the energy of HPTS fluorescence maxima versus normalized $E_T(30)$ is given in Fig. 2(b). For all points the correlation coefficient is 0.900. With the smaller data set the correlation coefficient is 0.938. As can be seen from Figs 2(a) and 2(b), correlations with Z and $E_T(30)$ give similar results; this can be expected since Z and $E_T(30)$ are correlated together with $r = 0.978$.[15] For HPTS, fits with Z were typically as good as or better than those with $E_T(30)$.

The original Z scale has the limitation that in highly polar solvents its solvatochromatic intermolecular charge transfer transition overlaps with the $\pi \rightarrow \pi^*$ transition of the pyridinium cation. $E_T(30)$ values were originally limited to non-acidic solvents owing to spectral changes arising from protonation of the betaine dye in acidic solvents.[15] However, both scales can be expanded by linear correlation with other parameters. Potentially, HPTS could be used to extend these scales owing to its solubility and relative stability in acidic solutions. In order to further investigate the suitability of HPTS as a solvent probe of strong acidic solvent environments,

correlations of fluorescence maxima with the Y_{OTs} parameter were performed, as described below; Y_{OTs} values are available for sulphuric acid solutions of concentrations 0%–70% by weight[24].

Since correlation coefficients of HPTS with Z and $E_T(30)$ for a variety of solvents are highly dependent upon the selection of data, the use of these correlations should be considered as an approximation. We plan to continue to expand our data set in order to determine which conditions, including solvent dryness, lead to the optimum data set for these evaluations. As described below, HPTS fluorescence also gives a better correlation for ethanol–water mixtures; in this correlation the problem of solvent dryness has been eliminated, since the percentage of water is known accurately.

The Winstein Y scale, originally based on rates of solvolysis of t-butyl chloride, is one of the earlier solvent polarity scales. It is a measure of solvent ionizing power.[15] Z values were originally proposed owing to difficulties in the measurement of Y in a variety of solvents.[25] Fluorescence maxima of HPTS correlate slightly better with Y values,[15] $r = 0.916$; however, the data set is limited owing to the lack of Y values. For the same set of data points for which Y is available

Table 3. Fluorescence data for mixtures

Acetonitrile–water mixtures		Ethanol–water mixtures		Sulphuric acid–water mixtures	
X_{MeCN}	Fluorescence max. (kcal mol^{-1})	X_{EtOH}	Fluorescence max. (kcal mol^{-1})	$X_{H_2SO_4}$	Fluorescence max. (kcal mol^{-1})
0.05	65.79	1.0	68.07	0.369	62.45
0.101	66.24	0.9	67.90	0.349	62.51
0.150	66.24	0.8	67.90	0.329	62.67
0.201	66.50	0.7	67.59	0.296	62.67
0.241	66.19	0.6	67.59	0.213	63.34
0.250	66.24	0.5	67.59	0.155	63.56
		0.4	67.36	0.130	63.59
Correlations		0.3	67.24	0.109	63.76
$E_T(30)$, $r = 0.719$		0.2	67.10	0.0732	63.82
Y_{OTs}, $r = 0.728$				0.0443	63.93
		Correlations E_T^N, $r = 0.940$		0.0206	64.05

Correlations E_T^N, $r = 0.940$

Y_{OTs} $r = 0.980$, SD = 0.254
$Y_{OTs} = (242.27 \pm 18.76)$
     $- (3.587 \pm 0.278)E_{HPTS}$ (kcal mol^{-1})

Y, $r = 0.981$, SD = 0.269
$Y = (265.46 \pm 19.90) -$
     $(3.929 \pm 0.294)E_{HPTS}$ (kcal mol^{-1})

Correlation
Y_{OTs}, $r = 0.991$, SD = 0.0963
$Y_{OTs} = (77.20 \pm 3.16) -$
     $(1.139 \pm 0.050)E_{HPTS}$ (kcal mol^{-1})

Z, $r = 0.979$, SD = 0.726
$Z = (760.38 \pm 53.62) -$
     $(10.00 \pm 0.79)E_{HPTS}$ (kcal mol^{-1})

(nine points), the normalized $E_T(30)$ correlation coefficient was 0.863 and the Z correlation coefficient was 0.805. In order to investigate the correlation further, normalized $E_T(30)$,[26] Y_{OTs},[18] Y[18] and Z (obtained by extrapolation of data in Ref. 25) values were compared for ethanol–water mixtures (a data set of nine points); for acetonitrile–water mixtures (a data set of six points), normalized $E_T(30)$[26] and Y_{OTs}[18] correlations were compared; and for sulphuric acid–water mixtures (a data set of 11 points), Y_{OTs}[24] values were correlated. Data are presented in Table 3. As can be seen, where other values are available, Y correlation coefficients are the same or higher.

The Y_{OTs} scale, which is similar to the Y scale, is based on solvolysis of 2-adamantyl tosylate.[18] 2-adamantyl tosylate was originally utilized because its structure prevents nucleophilic solvent participation in solvolysis. For the HPTS fluorescence data, Y_{OTs} has more consistent correlation coefficients than the other parameters. Therefore comparisons of the quality of the fits with those for other parameters depend on the choice of data set.

The fluorescence data of HPTS in ethanol–water mixtures correlate well with $E_T(30)$ ($r = 0.940$), Y ($r = 0.981$), Y_{OTs} ($r = 0.979$) and Z ($r = 0.979$), as shown in Table 3. While the latter correlations are very good, they are insufficient for extension of the parameter ranges.[16] (Repetition of these experiments at higher resolution may improve the correlation, since the energy range of the fluorescence data is narrow.)

For sulphuric acid mixtures, correlations of HPTS fluorescence with Y_{OTs} are excellent ($r = 0.991$; see Figure 3). Values of Y_{OTs} are affected by protonation of the substrate in H_2SO_4 concentrations of 70% or more.[24] However, the fluorescence of HPTS correlates well enough that it could be used to extend the Y_{OTs} scale. It is described by the equation

$$Y_{OTs} = (77.20 \pm 3.16)$$
$$- (1.139 \pm 0.050)E_{HPTS} \text{ (kcal mol}^{-1})$$

with a standard deviation of 0.0963. (However, HPTS cannot be left for extended periods of time in concen-
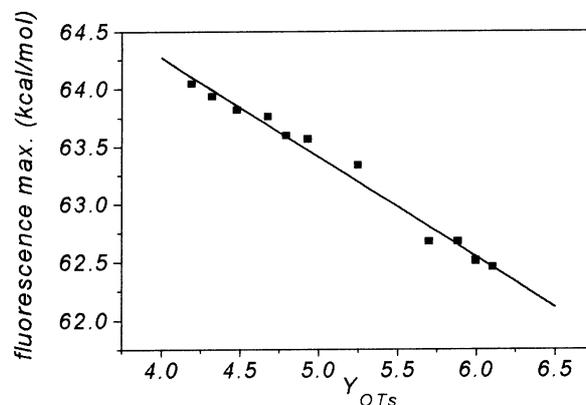
Figure 3. Correlation of HPTS fluorescence energy in sulphuric acid–water mixtures with Y_{OTs}

Table 4. Multi-parameter correlations

Correlation	Equation	Normalized coefficients[a]		Correlation coefficient[a]	
		Large data set	Small data set	Large data set	Small data set
Swain	$A = A_o + aA + bB$	$a = 0.914$ $b = 0.086$	$a = 0.872$ $b = 0.128$	0.904	0.951
Krygowski–Fawcett	$A = A_o + \alpha E_T(30) + \beta DN$	$\alpha = 0.793$ $\beta = 0.207$	$\alpha = 0.856$ $\beta = 0.144$	0.941	0.960
Kamlet–Taft	$A = A_o + s(\pi^* + d\delta) + a\alpha + b\beta$	$s = 6.02$ $a = 5.67$ $b = 6.06$ $d = -0.363$	$s = 5.67$ $a = 4.39$ $b = 3.77$ $d = -0.363$	0.873	0.991

[a] Data sets as indicated in Table 1.

trated sulphuric acid solutions, presumably owing to further sulphonation. Similar instability was noted in glacial acetic acid, presumably due to esterification.)

These results clearly indicate the utility of HPTS as a molecular probe of strong acidic solvent environments. In comparison with other probes, HPTS is unique because of its relative stability in acidic environments; in addition, it is readily available and safe and easy to use.

Multi-parameter correlations. A variety of multi-parameter approaches for the treatment of solvent effects are available. These methods utilize unique empirical parameters for distinct interaction mechanisms such as hydrogen bonding, electron pair donor–acceptor interactions and dipole–dipole interactions.[22] The results of three of these correlations with the HPTS fluorescence energy are given in Table 4.

The Swain A and B scale is a measure of a solvent's ability to solvate anions ('acity') and cations ('basity') respectively.[15] For HPTS the correlation coefficient was 0.904 and the parameter coefficients indicate that solvent acity has a greater effect on HPTS fluorescence.

The Krygowski–Fawcett correlation utilizes the $E_T(30)$ scale along with the Gutmann donor number (DN). These parameters measure solvent Lewis acidity and basicity respectively.[15] Once again, for HPTS fluorescence the correlation indicates that solvent Lewis acidity is more effective ($r = 0.941$, $E_T(30)$ coefficient $= 0.793$, DN coefficient $= 0.207$).

Kamlet–Taft correlations include separate parameters for solvent hydrogen bond donor acidity (α), hydrogen bond acceptor basicity (β), solvent dipolarity and polarizability (π^*) and a polarizability correction factor (δ). For HPTS the parameter coefficients from the correlation ($r = 0.873$) indicate that solvent hydrogen bond acidity/basicity and polarizability have a similar influence on the fluorescence spectra. Differences in the coefficients from multi-parameter fits may arise from the
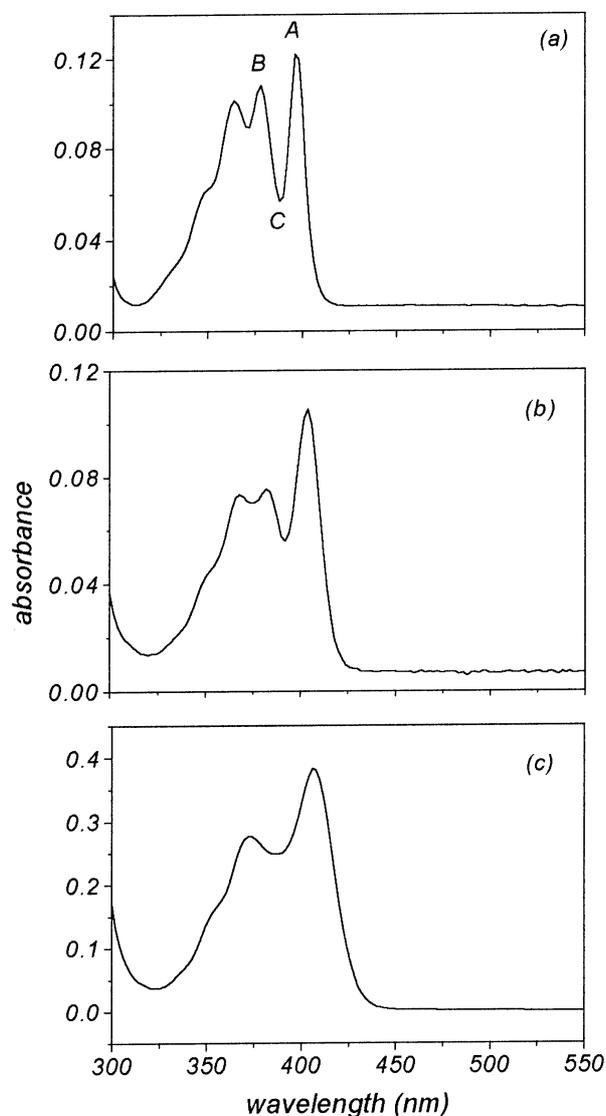
Figure 4. UV–visible absorption spectra of HPTS in (a) acetonitrile containing 1% methanol, (b) ethanol and (c) 30% HCl

separation of solvent polarizability in Kamlet–Taft fits; polarizability is included in the Swain B parameter[27] and in $E_T(30)$. We are continuing to study these and other results in order to gain a better understanding of the solvation of HPTS.

UV–visible absorption spectra

UV–visible absorption spectra for HPTS in acetonitrile (containing 1% methanol by volume), ethanol and 30 w/w% HCl are shown in Fig. 4. As can be observed, the relative position and heights of peaks A and B (the longest-wavelength bands) change with the solvent. In addition, the width of peak A varies. In order to compare spectral features, we calculated the ratio of the maximum of peak A to the longest-wavelength minimum (point C in Fig. 4(a)). This empirical measure appears to be sensitive to all three effects. Fluorescence of HPTS was compared with this ratio, as shown in Figs 5(a) and 5(b). The correlation coefficient for the entire data set was 0.939, while $r = 0.937$ with the smaller data set. From this correlation it is apparent that differential solvation of the ground and excited states of HPTS proportionally influences its UV–visible absorption and fluorescence spectra.[15]

Fluorescence versus UV–visible absorption curves were also plotted by grouping acidic and non-acidic solvents separately (see Fig. 5(b)); by doing this, the correlation coefficient improves for the non-acidic solvents to 0.974, while for acids it is 0.784. With exclusion of a point for acetic acid (with 0.5% methanol by volume) the correlation coefficient for acids becomes 0.900. From these results one can discern whether a solvent provides an environment that is more similar to that of an acidic solution or to that of a non-acidic solution.

In order to test whether the spectral shifts, in general, are primarily due to gradual protonation of the sulphonate groups, we have done preliminary fluorescence experiments on 1,3,6,8-pyrenetetrasulphonic acid tetrasodium salt in three solvents (methanol, water and concentrated sulphuric acid). The shifts observed for the 1,3,6,8-pyrenetetrasulphonate fluorescence are not as large as those observed for HPTS. For example, 1,3,6,8-pyrenetetrasulphonate has a fluorescence maximum of $\lambda = 394$ nm (72.6 kcal mol^{-1}) in sulphuric acid and 385 nm (74.3 kcal mol^{-1}) in methanol, which is a difference of 1.7 kcal mol^{-1}. In comparison, HPTS fluorescence maxima are at $\lambda = 461$ nm (62.0 kcal mol^{-1}) in sulphuric acid and 423 nm (67.6 kcal mol^{-1}) in methanol, corresponding to a much larger difference of 5.6 kcal mol^{-1}. From these results we may conclude that protonation of the sulphonate groups does not contribute significantly to the solvent-dependent fluorescence spectral changes seen with HPTS. In addition, the fluorescence data obtained in sulphuric acid solutions correlate excellently with Y_{OTS}, beyond the pH range where protonation of the sulphonate groups[2] could occur ($pK_a = 0–1$). The differ-
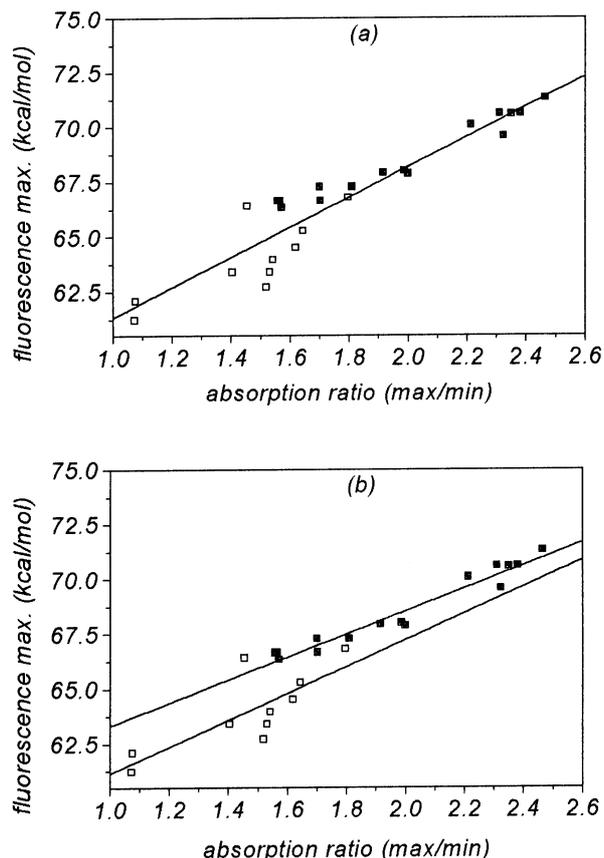
Figure 5. Fluorescence maxima versus UV–visible absorption ratios for HPTS in a variety of solvents. Full squares are non-acidic solutions and open squares are acidic solutions. Graph (a) has all data points included in the fit and graph (b) has acidic and non-acidic solutions fitted separately. Note that the acidic solutions which lie close to the non-acidic solution fit are for HPTS in acetic acid and acetic acid with 1% methanol by volume

ence in the above correlations of the HPTS spectral data in acidic and non-acidic solvents, therefore, appears to originate from small changes in the absorption spectra of HPTS. Apparently, the spectral changes observed are due to a unique combination of HPTS structural features, arising from the influence of the pyrene ring, the hydroxyl group and the sulphonate groups.

Correlation of HPTS UV–visible absorption data with

Table 5. Correlations of UV–visible absorption spectral data

Parameter	Data set[a]	Correlation coefficient
$E_T(30)$ normalized	Large	0.916
	Small	0.919
Z	Large	0.860
	Small	0.899
Y	Large	0.906
	Small	0.982

[a] Data sets as indicated in Table 1.
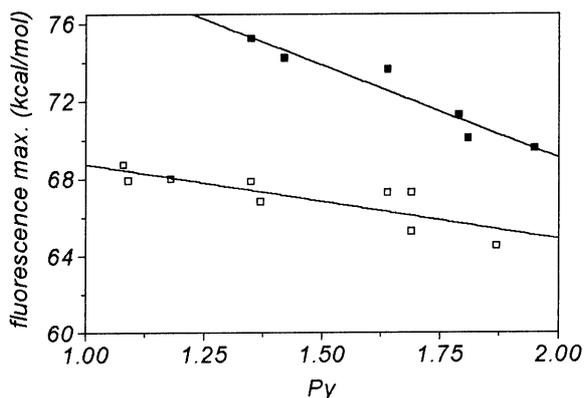
Figure 6. Correlation of HPTS fluorescence energy with *Py* for protic (open squares) and aprotic (full squares) solvents

polarity parameters yielded similar fits with Y ($r = 0.906$) and with $E_T(30)$ ($r = 0.916$). For the smaller data set, Y gave an excellent correlation ($r = 0.982$; however, there are only four points in the data set; see Table 5). We are continuing to explore the possibility that the UV–visible absorption of HPTS could be used as an additional measure of solvent polarity.

Additional categorization of solvents

Fluorescence data obtained in protic and aprotic solvents were studied by separation of the correlations obtained in the two types of solvents. (Absorption data could not be correlated separately, since only three data points are available for UV–visible absorption spectra of HPTS in neat, aprotic solvents owing to its low solubility.) For fluorescence data, best fits were obtained with the *Py* polarity parameter ($r = 0.961$, aprotics; $r = 0.830$, protics; see Table 2 and Fig. 6). The *Py* polarity parameter is based on relative emission intensities of the $\pi^* \rightarrow \pi$ band of pyrene. Similarities in solvation of pyrene and HPTS may give rise to the excellent correlation in aprotic solvents. Here too, as with the UV–visible absorption data described above, one can use the information in order to discern the type of environment in which the HPTS is found.

CONCLUSIONS

HPTS is a unique molecular probe. Not only can its photoacidic qualities be used to measure pH,[9,10] but its fluorescence shift and UV–visible absorption band changes can be used as measures of solvent polarity. HPTS is uniquely stable, and protonation of the sulphonate groups at pH values less than one[2] apparently does not greatly affect the continuity of correlations obtained.

The high correlation of HPTS fluorescence maxima in

sulphuric acid solutions with Y_{OTs} indicates the usefulness of HPTS as a polarity probe in acidic environments. In addition, the HPTS fluorescence shift can be used to determine whether a solvent environment is protic or aprotic in nature (when correlated with *Py*) or whether it resembles that of an acidic solution (when correlated with its UV–visible absorption ratio).

Multi-parameter correlations indicate that HPTS fluorescence is mainly influenced by the Lewis acidity and polarizability of the solvent of interest. UV–visible absorption and fluorescence spectra are affected by the unique solvation of the HPTS pyrene ring, sulphonate and hydroxyl groups.

EXPERIMENTAL

Materials

Solvents were typically the highest commercial grade available. HPTS was typically Kodak laser grade, and 1,3,6,8-pyrenetetrasulphonic acid tetrasodium salt was purchased from Molecular Probes. All materials were used as received.

Fluorescence

Spectra were recorded on one of two instruments. The first was an SLM Instruments Inc. fluorimeter with excitation at 340 nm and slit width varied in order to improve signal and resolution. The second was an SLM-Aminco-Bowman Series 2 luminescence spectrometer with excitation at 353 nm, slit widths of 4 nm, data collection every 1 nm and averaging of three spectra. Computer solvent corrections were performed with the second spectrometer when necessary; however, instrumental corrections were omitted. Owing to similarities in data, results obtained from both fluorimeters were averaged together. Experiments in sulphuric acid solutions were performed with higher resolution owing to the narrower range of energy values. Excitation was at 380 nm with 16 nm excitation bandpass and 0.5 nm emission bandpass; data were collected every 0.2 nm, eight spectra were averaged together and an instrument correction was performed.

UV–visible absorption spectra

UV–visible absorption experiments were performed using a Hewlett-Packard 8452A diode array spectrophotometer. Experiments were performed either with solvent blank subtraction or with estimation of the solvent baseline. Results from both methods were averaged together.

Errors

Standard deviations for the spectroscopic measurements are illustrated by the following examples. UV–visible absorption and fluorescence spectra of HPTS in acetone (containing 1:50 v/v methanol) and in water (containing acid) were each measured nine times. Standard deviations for the HPTS fluorescence maxima were ± 0.14 kcal mol^{-1} with the acetone mixture as the solvent and ± 0.11 kcal mol^{-1} with water as the solvent. Absorption ratios had standard deviations of ± 0.097 (acetone mixture) and ± 0.024 (water). Factors contributing to the error include HPTS solubility and sample concentration.

Errors indicated for correlations are standard errors of fit with equal weighting for all points.

Data analysis

One-parameter fits and interpolation of literature polarity parameters for mixtures were performed using the Origin Microcal 4 plotting package. Polarity parameter interpolations were either cubic or linear, depending upon the quality of the fit. Multi-parameter correlations were performed using Matlab 4 software, written by Dr. D. Pines.

Acknowledgements

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