

SOLVENT EFFECTS ON ELECTRONIC ABSORPTION AND FLUORESCENCE SPECTRA OF SALICYLALDEHYDE 2-PYRIDYLHYDRAZONE AND INDOLALDEHYDE 2-SALICYLHYDRAZONE: GROUND AND EXCITED STATE DISSOCIATION CONSTANTS

M. ROMÁN CEBA, C. CRUCES BLANCO and F. GARCÍA SÁNCHEZ

Department of Analytical Chemistry, Faculty of Science, The University, Málaga 29004 (Spain)

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Summary

The absorption and fluorescence spectra of salicylaldehyde 2-pyridylhydrazone (SAPH) and indolaldehyde 2-salicylhydrazone (IASH) were studied in various solvents. The relative fluorescence intensity and efficiency ratio are reported. Highly polar solvents cause red shifts in SAPH and blue shifts in IASH which indicate that the long-wavelength bands of the neutral molecule arise from $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions respectively.

The ground state dissociation constants pK_a of SAPH and IASH were measured spectrophotometrically and spectrofluorometrically in 40 vol.% ethanol-water solutions. The corresponding pK_a reveal that the compounds have an increased acidity in the excited state.

1. Introduction

Solvent effects on molecular spectra are often viewed as selective spectroscopic analysis because they induce significant changes in the position, intensity and shape of the absorption and fluorescence bands [1]. Solvation studies are of interest in analytical spectroscopy because the information obtained can be used to design more selective spectroscopic measurements in such a manner that one of the most important features of the planned reaction is the selection of a suitable solvent.

The changes in the excitation and emission wavelengths with changes in the polarities of the solvents depend on whether S_1 or S_0 is more stabilized by the solvent. In $\pi \rightarrow \pi^*$ transitions S_1 is often more polar than S_0 , so that on increasing the solvent polarity λ_{em} and λ_{ex} undergo red shifts. The inverse relationship is often true for $n \rightarrow \pi^*$ transitions. In contrast, the energy differences between the Franck-Condon ground states can be related to the Stokes shift [2].

Although the excited singlet state is very short lived, the occurrence of proton exchange between photoexcited organic molecules (including metallic chelates) is very frequent because many drugs, pesticides, dyes and other molecules [3] have acidic or basic functional groups. Therefore acid excited states (and ground states) undergo reactions that can cause serious problems in analytical fluorometry or, conversely, can provide a way of improving the analytical characteristics of the method. Consequently, the well-informed analyst must be aware of the ways in which the motion of excited protons can influence the fluorescence spectra of the materials being analysed.

A study of solvent effects and acid-base behaviour in salicylaldehyde 2-pyridylhydrazone (SAPH) and indolaldehyde 2-salicylhydrazone (IASH) is described in this paper. These organic ligands are of potential interest in analytical chemistry: SAPH has been used in the determination of magnesium [4] and aluminium [5], and IASH is used to determine magnesium, strontium and barium [6].

2. Experimental details

2.1. Materials

SAPH and IASH were synthesized using the standard method for hydrazones [7] by heating equimolecular amounts of salicylaldehyde and indolaldehyde with pyridylhydrazine and salicylhydrazine respectively. The starting materials were obtained from Aldrich Chemicals. All solvents used were of analytical grade.

2.2. Apparatus

The absorption spectra and absorptiometric data were obtained using a Shimadzu UV-240 Graphicord spectrophotometer.

The fluorometric measurements were performed using a model MPF-43A Perkin-Elmer fluorescence spectrophotometer equipped with an Osram XBO 150 W xenon lamp. An ultrathermostatic water bath circulator (Frigiterm S-382) was used to control the solution temperature at 25 ± 0.1 °C.

All pH measurements were performed using a Digit 501 Crison pH meter with a combined electrode.

2.3. Titration procedure

1×10^{-5} M solutions of SAPH or IASH in 250 ml of a 40vol.% ethanol-water mixture were prepared. In each fluorometric or photometric titration and aliquot of 3 ml of the reagent solution, which had previously been adjusted to the desired pH by adding small amounts of dilute NaOH or HCl, was placed in the measurement cell. No corrections for ionic forces were applied to the titration data because the solution concentrations at $pK_a \pm 2$ were less than 10^{-4} M for acid, base and reagent. All measurements were made at 25 °C.

3. Results and discussion

3.1. Solvent effects on spectral data

In order to evaluate the effect of solvents of various polarities and hydrogen-bond-forming capacities on the fluorescence and absorption spectra, an experiment was carried out in which the spectra of 1×10^{-5} M solutions of SAPH and IASH in each reagent were recorded. The results are shown in Tables 1 and 2.

As can be seen in Table 1, the absorption spectrum of SAPH (band maximum) is slightly blue shifted on going from dioxane to acetonitrile. However, the red shift and the Stokes shift (absorption maximum to fluorescence maximum) calculated for the fluorescence spectrum from the data of Table 1 increase with increasing solvent polarity, and this indicates that the long-wavelength band of the neutral molecule is due to $\pi \rightarrow \pi^*$ transitions.

The hydrogen-bonding capacity of the solvents must also be taken into consideration because it could be more important than the polarity. This is shown by the anomalous behaviour of acetonitrile which is more polar than dimethylformamide but is exclusively a hydrogen-bond acceptor.

TABLE 1

Spectral characteristics of salicylaldehyde 2-pyridylhydrazone in various solvents

<i>Solvent</i>	<i>Dielectric constant</i>	λ_{abs} (nm)	λ_f (nm)	$\Delta\lambda$ (nm)	$\log \epsilon$	RFI	$\text{ER} \times 10^{-3}$
Dioxane	2.25 (20 °C)	337	370	33	4.37	0.41	0.17
Chloroform	4.81 (20 °C)	335	—	—	4.42	—	—
Acetone	20.70 (25 °C)	337	390	53	4.40	0.13	0.05
Ethanol	24.30 (25 °C)	335	440	105	4.34	54.00	24.40
Methanol	32.70 (25 °C)	335	440	105	4.45	27.00	9.47
Dimethylformamide	36.71 (25 °C)	340	450	110	4.39	87.00	35.10
Acetonitrile	37.50 (20 °C)	332	450	118	4.46	3.40	1.16

TABLE 2

Spectral characteristics of indolaldehyde 2-salicylhydrazone in various solvents

<i>Solvent</i>	<i>Dielectric constant</i>	λ_{abs} (nm)	λ_f (nm)	$\Delta\lambda$ (nm)	$\log \epsilon$	RFI	$\text{ER} \times 10^{-3}$
Dioxane	2.25 (20 °C)	337	435	98	3.95	48	53
Chloroform	4.81 (20 °C)	335	465	130	4.43	67	25
Acetone	20.70 (25 °C)	333	410	77	4.39	22	9
Ethanol	24.55 (25 °C)	336	430	94	4.49	72	23
Methanol	32.70 (25 °C)	335	430	95	4.37	26	11
Dimethylformamide	36.71 (25 °C)	327	420	93	4.15	880	617
Acetonitrile	37.50 (20 °C)	332	432	103	4.28	26	13

As can be seen in Table 1 it causes a decrease in the relative fluorescence intensity RFI and efficiency ratio ER but shows the greatest Stokes shift and the highest molar absorptivity.

These results indicate that, although the probability of absorption transition is greater in polar solvents, this is not the case for the fluorescence intensity because, as shown by the efficiency ratio, other processes such as internal conversion and intersystem crossing which compete with fluorescence are favoured by the greater hydrogen-bond-acceptor character of acetonitrile relative to other polar and hydrogen-bond-donor solvents such as ethanol which inhibit these non-radiative processes. Therefore it can be deduced that the best results are obtained in solvents with high dielectric constants such as ethanol or dimethylformamide where the fluorescence transition is favoured by hydrogen-bond interactions between the solute and the solvent.

Table 2 shows the spectral characteristics of IASH in various solvents. Its behaviour is opposite to that of SAPH, and a blue shift is observed with increasing solvent dielectric constant. This blue shift has been used to characterize $n \rightarrow \pi^*$ transitions and to distinguish them from $\pi \rightarrow \pi^*$ transitions. This criterion was suggested by McConnell [8]. The blue shift criterion is observed in both the absorption maxima, where it is about 5 nm more for dioxane than for acetonitrile, and the fluorescence maxima. However, the RFI and ER values exhibit some anomalous behaviour.

In general the largest values of the RFI and ER are obtained for solvents with small dielectric constants such as dioxane and chloroform, but larger values were obtained in ethanol than in acetone or acetonitrile because ethanol is less polar than the other two solvents. The presence of a lone pair of electrons on a relatively electronegative nitrogen atom in this $n \rightarrow \pi^*$ transition suggests strongly that a specific hydrogen-bonded complex is formed between a hydroxylic solvent such as ethanol and the solute.

The most unusual behaviour of the seven solvents studied is exhibited by dimethylformamide which produces the greatest blue shift with respect to dioxane (15 nm) and the largest RFI and ER despite having one of the highest dielectric constants. This indicates that some other factor must be taken into account to explain the anomalous behaviour of this solvent. This factor is the strong polarity of its hydrogen bond character, and therefore it is known as a polar aprotic solvent. Therefore the phenomena competing with fluorescence are minimized in this type of solvent.

To corroborate the type of electronic transition assigned to each compound, a study based on the work of Kosower [9, 10] has been carried out. Kosower defined values Z which are standard measures of solvent polarity and are related to the transition energy by the equation

$$E_T = 2.859 \times 10^{-3} \bar{\nu}$$

where $\bar{\nu}$ is in reciprocal centimetres. If the slope obtained when this equation is applied to a particular compound is negative, the band can be attributed

to a $\pi \rightarrow \pi^*$ transition, whereas if the slope is positive an $n \rightarrow \pi^*$ transition has taken place.

Linear regression analysis gives the equations

$$E_T = -0.1Z + 100$$

for SAPH and

$$E_T = 0.02Z + 84.55$$

for IASH which correspond to the previous results.

3.2. Ground state pK values

In order to obtain ground state pK values detailed absorptiometric and fluorometric studies were performed to determine the effect of acidity on the electronic spectra of the compounds. For this purpose a photometric and fluorometric titration was performed for each reagent. The absorption spectra of SAPH and IASH for a wide range of pH values are shown in Figs. 1 - 3.

The ground state dissociation constants of the two reagents were calculated using the analytical method of Pease and Williams [11]. This method is based on the standard formula

$$K_a = \frac{A - A_{BH^+}}{A_B - A} [H^+]$$

where A_{BH^+} and A_B are the absorbances of the acidic (non-dissociated) and basic (dissociated) forms of the molecule respectively. The absorbance

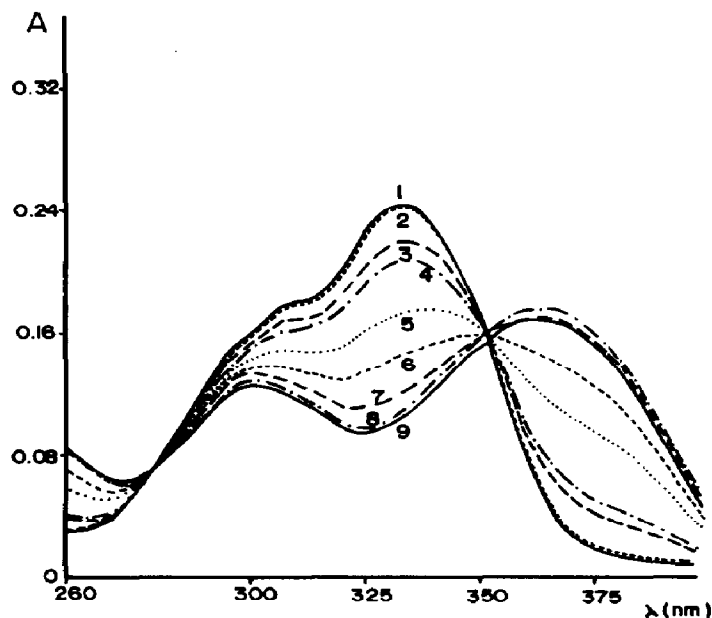


Fig. 1. Absorption spectra of SAPH at various pH values (1×10^{-5} M SAPH in 40vol.-%-ethanol-water): curve 1, 9.36; curve 2, 9.52; curve 3, 10.21; curve 4, 10.60; curve 5, 11.23; curve 6, 11.55; curve 7, 12.10; curve 8, 12.56; curve 9, 12.69.

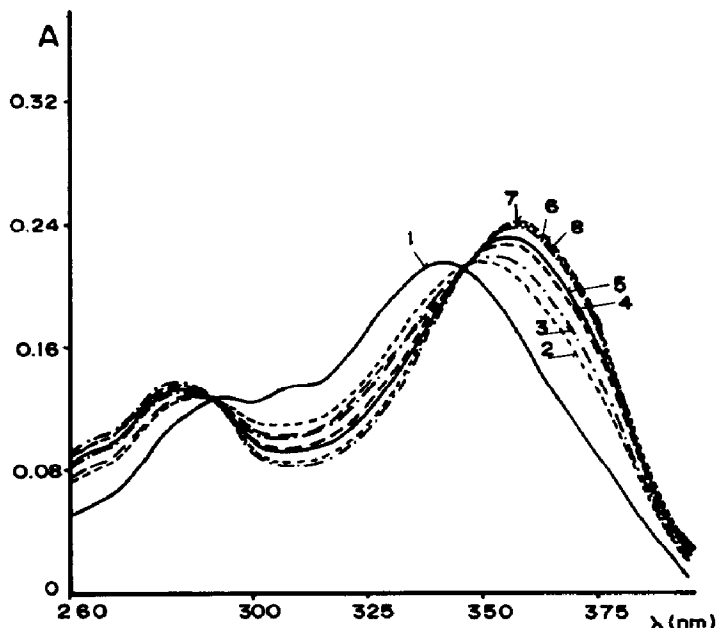


Fig. 2. Absorption spectra of SAPH at various pH values (1×10^{-5} M SAPH in 40vol.-%-ethanol-water): curve 1, 4.88; curve 2, 4.40; curve 3, 4.28; curve 4, 3.98; curve 5, 3.81; curve 6, 3.04; curve 7, 2.49; curve 8, 2.20.

values used to calculate pK_a were measured at a wavelength where a small change in the pH values causes a large change in the absorbance. This wavelength was 370 nm for SAPH and 360 nm for IASH. The pK_a values obtained for each reagent using the method of Pease and Williams are summarized in Table 3.

Fluorescence spectroscopy can be used in the same manner as absorption spectroscopy to determine the ground state dissociation constants, but the analytical excitation wavelength must be carefully chosen [12].

Therefore the choice of wavelength at which the excitation of both acid and basic forms takes place at proton exchange equilibrium can affect the partial contribution of excited state prototropism, and this is why the pK_a values for absorptiometry and fluorometry differ slightly from each other in most cases. This can be avoided by the selective excitation of either the acid or the basic form of the compound. Consequently, it is possible to study ground state prototropism although the excited state dissociation can also take place.

This treatment has been applied to SAPH (selective excitation at $\lambda = 378$ nm) and IASH (selective excitation at $\lambda = 365$ nm). The equation used was

$$K_a = \frac{(F - F_A^\circ)[H^+]}{F_B^{\text{const}} - F}$$

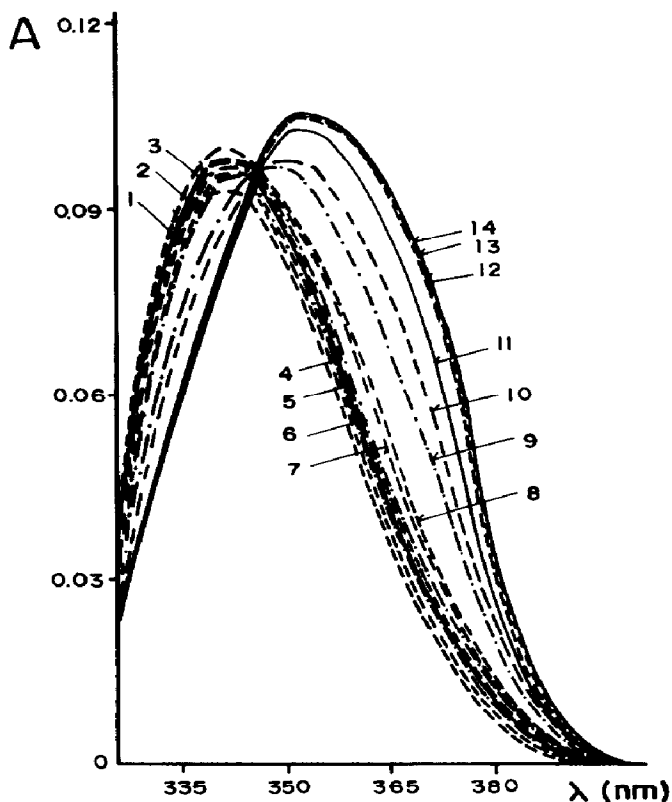


Fig. 3. Absorption spectra of IASH at various pH values (1×10^{-5} M IASH in 40vol.-% ethanol-water): curve 1, 2.95; curve 2, 3.76; curve 3, 5.00; curve 4, 5.55; curve 5, 6.67; curve 6, 6.80; curve 7, 7.57; curve 8, 8.03; curve 9, 8.62; curve 10, 8.68; curve 11, 9.57; curve 12, 10.59; curve 13, 11.04; curve 14, 11.76.

TABLE 3

Ground state pK_a values of salicylaldehyde 2-pyridylhydrazone

Technique	pK_{a_1} ^a	pK_{a_2} ^a
Spectrophotometry		
SAPH, $\lambda = 370$ nm	4.85 ± 0.07 (6)	10.98 ± 0.37 (5)
IASH, $\lambda = 360$ nm	8.30 ± 0.19 (5)	—
Spectrofluorometry		
Selective excitation of basic form:		
SAPH, $\lambda_{ex} = 385$ nm; $\lambda_{em} = 440$ nm	—	10.55 ± 0.09 (5)
IASH, $\lambda_{ex} = 365$ nm; $\lambda_{em} = 430$ nm	8.37 ± 0.17 (3)	—
Excitation at isosbestic point:		
SAPH, $\lambda_{ex} = 355$ nm; $\lambda_{em} = 440$ nm	4.86 ± 0.48 (5)	10.40 ± 0.12 (5)
IASH, $\lambda_{ex} = 345$ nm; $\lambda_{em} = 430$ nm	8.46 ± 0.21 (3)	—

^aThe numbers in parentheses are the numbers of determinations.

where F_A° is the fluorescence intensity when the solution is so acidic that all the compound is in the protonated form,

$$F_B^{\text{const}} = F_B^\circ \left(\frac{\phi_B}{\phi_B^\circ} \right)_{\text{const}}$$

where ϕ_B and ϕ_B° are the fluorescence quantum efficiency of the basic form in the occurrence and in the absence respectively of proton exchange in the lowest excited singlet state and F is the fluorescence intensity at any pH value. This is the first case in which possible proton exchange in the excited state is taken into account. To avoid the possibility of proton exchange in the excited state during the determination of pK_a , the excitation was performed at isosbestic points in the absorption spectra of SAPH ($\lambda = 355 \text{ nm}$) and IASH ($\lambda = 345 \text{ nm}$). The data obtained for each excitation of both reagents are given in Table 3.

It can be seen that two values of pK_a are obtained for SAPH ($pK_{a_1} = 4.85 \pm 0.07$ and $pK_{a_2} = 10.98 \pm 0.37$) which, based on other data for N-heterocyclic bases [13], can be assigned to the deprotonation of pyridinic hydrogen and the hydrogen from the phenolic OH group respectively. The schemes for these processes are shown in Fig. 4.

A spectroscopic scan over the pH range 2 - 8 in SAPH, *i.e.* the region of existence of the conjugated acid form BH, showed no fluorescence. Consequently, $F_A^\circ = 0$ in the equation given above which then becomes

$$K_a = \frac{F[H^+]}{F_B^{\text{const}} - F}$$

This indicates that pK_{a_1} can be calculated for SAPH, regardless of the excitation conditions, by recording the fluorescence spectrum at low pH. The

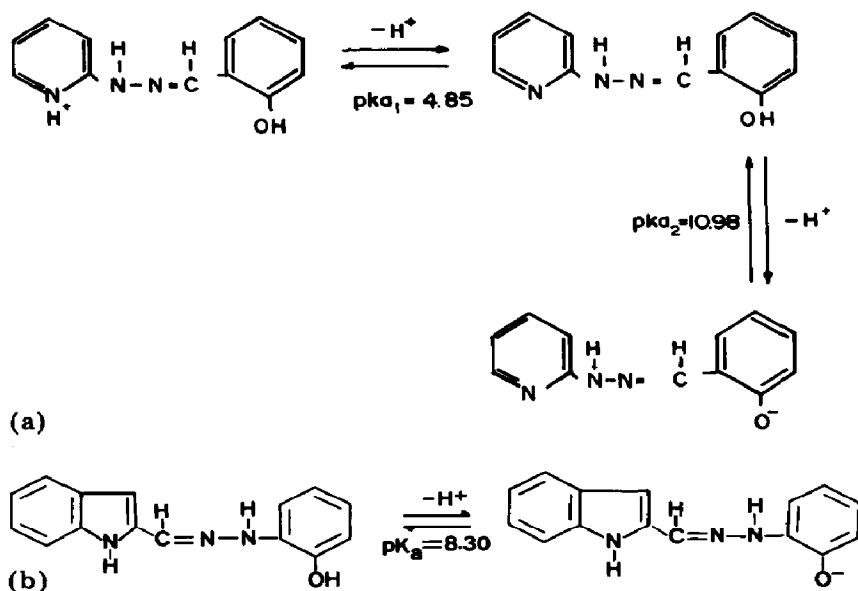


Fig. 4. Scheme of the dissociation process for (a) SAPH and (b) IASH.

ground state pK_{a_1} values were determined spectrofluorometrically and spectrophotometrically using the method of Pease and Williams [11] and were found to be 4.86 ± 0.48 and 4.85 ± 0.07 respectively.

Only one ground state pK_a value (8.37 ± 0.08) is obtained for IASH and this is assigned to the loss of a proton from the phenolic OH group. This value is a mean of those obtained using spectrophotometry and spectrofluorometry where the data are very close, indicating an absence of proton exchange in the excited state of the compound.

3.3. Excited state pK_a values

The excited state pK_a values of SAPH and IASH were calculated from the Förster cycle [14, 15] using the equation

$$pK_a - pK_a^* = 2.1 \times 10^{-3}(\bar{\nu}_{BH^+} - \bar{\nu}_B)$$

where pK_a and pK_a^* are the ground and excited state dissociation constants respectively and $\bar{\nu}_{BH^+}$ and $\bar{\nu}_B$, expressed in reciprocal centimetres, are the wavenumbers of the absorption or fluorescence transitions of the protonated molecule BH and the free base B respectively. The data required for this calculation and the results obtained are given in Table 4.

Although fluorometric titration is a well-established method of calculating pK_a^* , it has not been used here because the prototropic equilibrium is not attained during the lifetimes of the excited species, *i.e.* the lifetime of the molecule in the lowest excited state is so short that it impedes prototropism in the excited state.

As can be seen, the values obtained for the excited state dissociation constants of SAPH and IASH are smaller than the ground state values which indicates that both compounds are weaker bases in the excited state than in the ground state. The difference of 2.32 pK_a units in SAPH and 1.51 pK_a units in IASH between the absorption and fluorescence data could be due to solvent relaxation and/or using the band maxima of the species rather than 0-0 transitions.

TABLE 4
Spectral data and Förster cycle calculations for SAPH and IASH

		Absorption	Fluorescence	Average
$\lambda_{\max}(\text{BH})$ (nm)	SAPH	364	438	401
	IASH	330	420	375
$\lambda_{\max}(\text{B})$ (nm)	SAPH	378	442	410
	IASH	345	430	387.5
$\Delta\bar{\nu}$ (cm^{-1})	SAPH	1017.5	188.62	603.06
	IASH	1317.5	553.70	935.60
pK_a	SAPH	10.98	10.40	10.67
	IASH	8.30	8.41 ^a	8.35
pK_a^*	SAPH	8.84	9.97	9.40
	IASH	5.53	7.25	6.39

^aAverage of two excitations.

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