

## PHOTOPHYSICAL PROPERTIES OF 1,8-NAPHTHALIMIDE DERIVATIVES

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### Summary

A study of the photophysical properties of the derivatives of 1,8-naphthalimide is presented. The absorption and fluorescence spectra in protic and aprotic media are consistent with the assumption of exciplex formation. The application of the technique of single-photon counting was of great importance for the lifetime measurements. The exciplex formation is interpreted as resulting from proton migration.

### 1. Introduction

1,8-Naphthalimide derivatives, such as brilliant sulphoflavine and lucifer yellow, are colourings used as both laser dyes and fluorescent cell markers [1, 2]. Other derivatives from the same parent compound have been studied as laser dyes [3]. Recent studies have shown that some 1,8-naphthalimide derivatives have antitumour properties [4, 5].

These characteristics suggest that the photophysical properties of these substances should be studied in depth. In this paper, studies of the absorption and fluorescence spectra in different media are described, together with those of the effect of the presence of protons in an aprotic medium (acetonitrile). These studies were based on the measurement of lifetimes by the technique of photon counting. In order to carry out the study systematically, different substituents were used at the imide group ( $R_1$ ) and at position 3 of the naphthalic ring ( $R_2$ ).

### 2. Experimental procedure

The 1,8-naphthalimide derivatives used have the substituents  $R_1$  and  $R_2$  indicated in Table 1. The structure of these compounds is shown in Fig. 1.

These derivatives were synthesized by the method described by Braña and coworkers and were obtained in crystalline form [6]. The imide of the

TABLE 1

Naphthalimide derivatives used in this study: the substituents R1 and R2

Derivative	R <sub>2</sub>	R <sub>1</sub>
1,8-Naphthalimide I	-H -NH <sub>2</sub>	-H -CH <sub>2</sub> -CH <sub>2</sub> -N(CH <sub>3</sub> ) <sub>2</sub>
II	-NH <sub>2</sub>	-CH <sub>2</sub> -CH <sub>2</sub> -N(CH <sub>2</sub> -CH <sub>3</sub> ) <sub>2</sub>
III	-O-CH <sub>3</sub>	-CH <sub>2</sub> -CH <sub>2</sub> -N(CH <sub>2</sub> ) <sub>2</sub>
IV	-NO <sub>2</sub>	-CH <sub>2</sub> -CH <sub>2</sub> -N(CH <sub>2</sub> ) <sub>2</sub>
V	-NO <sub>2</sub>	-CH <sub>2</sub> -CH <sub>2</sub> -N(CH <sub>3</sub> ) <sub>2</sub>
VI	-NH <sub>2</sub>	-CH <sub>2</sub> -CH <sub>2</sub> -N(CH <sub>2</sub> ) <sub>2</sub>

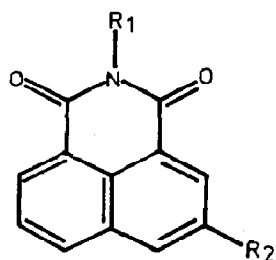


Fig. 1. Structure of the naphthalimide derivatives.

naphthalic acid was purified by recrystallization in water. The solvents used were of Uvasol quality and the water was distilled three times.

The absorption spectra were recorded using a Cary 17 spectrophotometer. For the fluorescence spectra a Hewlett-Packard spectrofluorometer was used. The fluorescence decay curves were measured with a computerized Applied Photophysics nanosecond spectrometer. The fluorescence decay data were fitted to a single exponential function by means of an iterative deconvolution program, taking as statistical criteria the value of  $\chi^2$  and the Durban-Watson parameter, and likewise for the analysis of the residuals. Preliminary results were reported at EUCMOS XVII [7].

### 3. Results

#### 3.1. Absorption spectra

The absorption spectra in the region 300 - 500 nm (normalized to the maximum) corresponding to 1,8-naphthalimide and the derivatives V, III and I with the substituents  $R_2 \equiv \text{NO}_2$ ,  $\text{O}-\text{CH}_3$  and  $\text{NH}_2$  are presented in Fig. 2 (acetonitrile was used as the solvent). Spectra were also obtained in other solvents, such as methanol, ethanol, dimethyl sulphoxide and water.

The effect on the behaviour of the visible band on introducing protons into the acetonitrile, by adding small quantities of sulphuric acid solution, is now discussed.

For derivatives with  $R_2 \equiv \text{NH}_2$  a slight decrease in the intensity of the transition and a red shift of the maximum are observed. This band disappears if the acid concentration is greatly increased.

The derivatives with  $R_2 \equiv \text{O}-\text{CH}_3$  present a slight decrease in the intensity of the 380 nm band and the derivatives with  $R_2 \equiv \text{NO}_2$  show practically no variation owing to the low intensity of the visible band.

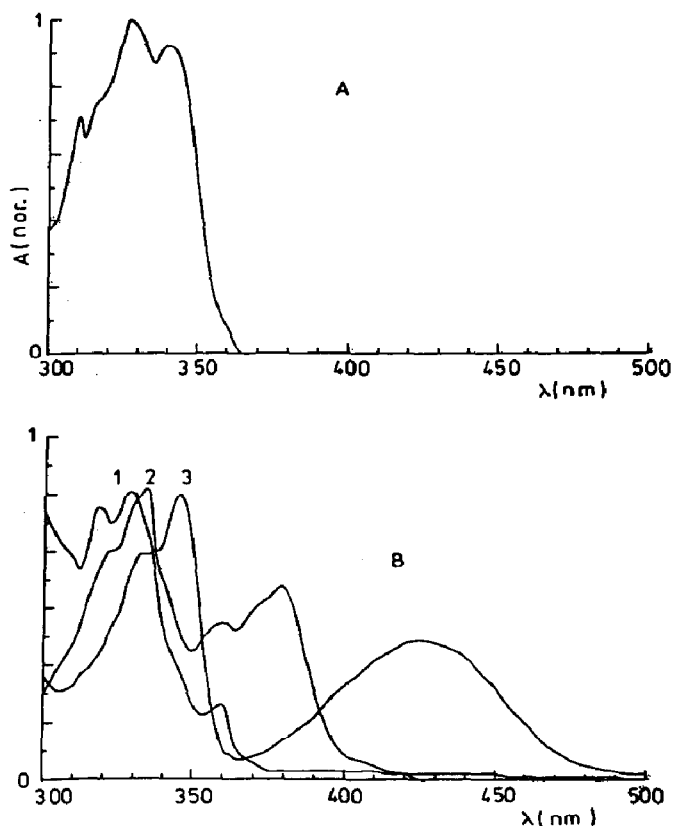


Fig. 2. Absorption spectra. (A) 1,8-naphthalimide. (B) Derivatives: curve 1, V; curve 2, III; curve 3, I.

### 3.2. Fluorescence spectra

The fluorescence spectra of compounds with  $R_2 \equiv NH_2$  possess the following characteristics. In a solvent such as acetonitrile (characterized as an aprotic medium) the fluorescence is the mirror image of the absorption. Thus the absorption maximum is at 425 nm and the fluorescence maximum is at 510 nm.

In solvents with acid-base character such as water, ethanol and methanol the fluorescence appears to be displaced from the mirror position. Thus the absorption maximum in these solvents is at 425 nm (similar to that in acetonitrile) but the fluorescence maximum appears between 550 and 570 nm.

Mixing small quantities of 0.1 N sulphuric acid with acetonitrile produces a marked fluorescence shift towards the red, with an appreciable increase in intensity, as shown in Fig. 3. This effect is highly pronounced for VI, but also occurs for I and II.

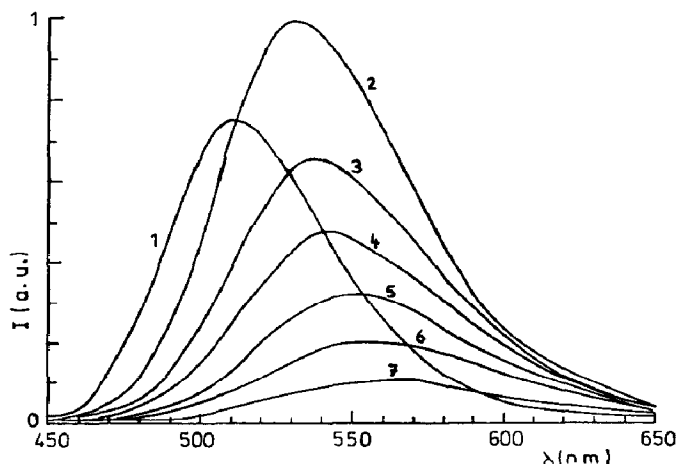


Fig. 3. Behaviour of the fluorescence spectrum of compound I in acetonitrile on the addition of small quantities of 0.1 N  $H_2SO_4$ . (Curve 1, in acetonitrile; curves 2 - 7, successive addition of 0.1 N  $H_2SO_4$ .)

IV and V, which have an  $NO_2$  substituent, present a very weak fluorescence in the region 500 - 520 nm, consistent with the weak absorption. Protonation does not have any detectable effect.

III, which has an  $O-CH_3$  substituent, shows fluorescence in acetonitrile, with its maximum at 415 nm, and is much less intense than that presented by the derivatives containing the  $NH_2$  group. This fluorescence is the mirror image of the absorption (absorption maximum is at 380 nm). On adding a small quantity of 0.1 N sulphuric acid there is a spectacular increase in the fluorescence intensity and a fluorescence red shift occurs simultaneously. If more sulphuric acid solution is added, the fluorescence is maintained; even if concentrated sulphuric acid is added, it does not disappear, as happens in the case of the derivatives containing  $NH_2$ .

### 3.3. Lifetime measurements

In order to delve deeply into the photophysical behaviour of the derivatives of 1,8-naphthalimide, we studied the variation in the fluorescence lifetime under different conditions.

It was only possible to study the lifetime of those derivatives with  $R_2 \equiv \text{NH}_2$  (these compounds are strongly fluorescent). The derivatives with  $\text{O}-\text{CH}_3$  and  $\text{NO}_2$  have such weak fluorescence that they give practically no measurable signal.

The lifetime of compound I varies according to the solvent used and is 7 ns in acetonitrile, and 11 ns, 13 ns and 12.5 ns in methanol, ethanol and dimethyl sulphoxide respectively. Changing  $R_1$  while retaining the same  $R_2$  ( $\text{NH}_2$ ) also has a significant effect on the lifetime; thus, in acetonitrile compounds I, VI and II have lifetimes of 7 ns, 3 ns and shorter than 2 ns respectively. On adding a small quantity of 0.1 N sulphuric acid solution ( $0.05 \text{ cm}^{-3}$  per  $10 \text{ cm}^3$  acetonitrile solution) to these three compounds in acetonitrile, the lifetimes are increased considerably, reaching 20 ns, 16 ns and 12 ns respectively. If addition of acid continues the lifetime slowly diminishes. If, instead of acid, only water is added, there is an increase in the lifetime which reaches a maximum and then decreases. These results are shown in Fig. 4.

The derivative with  $R_2 \equiv \text{O}-\text{CH}_3$  is only very weakly fluorescent in acetonitrile, making it impossible to measure its lifetime, but it shows a strong increase in fluorescence on adding a small quantity of acid as explained previously, permitting measurement of its lifetime under these conditions and giving a value of 3 ns. The lifetime does not vary on continuing the addition of acid.

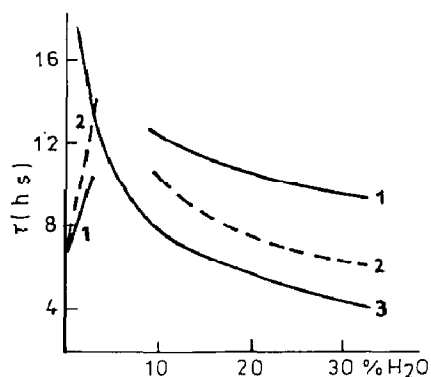


Fig. 4. Lifetime variation with water content. Curve 1, pure water; curve 2, 0.05 N  $\text{H}_2\text{SO}_4$ ; curve 3, 0.1 N  $\text{H}_2\text{SO}_4$ .

## 4. Discussion

The behaviour of the fluorescence and the lifetime in different solvents and the effect of the introduction of protons in acetonitrile were interpreted in the following manner.

The mirror fluorescence in the aprotic solvent acetonitrile and the marked fluorescence shift in protic media (water, methanol, ethanol and acidified acetonitrile) can be explained in terms of exciplex formation in the protic media. The exciplex is formed from the excited state of the 1,8-naphthalimide derivatives and a proton. Only in pure acetonitrile is exciplex formation impossible and in this case the fluorescence spectrum is the mirror image of the absorption spectrum. In protic media the excited state protonation is the main feature. This can be represented by the potential curves shown in Fig. 5. In pure acetonitrile the left-hand side of this figure is applicable while in acidified acetonitrile or in protic media the right-hand side is applicable. This explains both the observed red shift of the fluorescence on introducing protons in acetonitrile and the non-mirror fluorescence in protic media. The exciplex does not have a non-radiative channel, but one does exist for the non-protonated excited species.

These conclusions are reflected by the variations in the fluorescence intensity and the lifetime for solutions of compounds containing the substituents  $\text{NH}_2$  and  $\text{O}-\text{CH}_3$  (on adding small quantities of acid) and also by the behaviour in protonic solutions compared with that in pure acetonitrile solution.

Other measurements support these conclusions. The quantum yield of III goes from an almost negligible value in pure acetonitrile to a value of about 0.3 in weakly acidified acetonitrile. Similarly, the quantum yield of VI goes from 0.1 to 0.45.

Another finding is that the lifetimes and quantum yields of derivatives containing  $\text{NH}_2$  diminish with the addition of greater quantities of acid (more than  $0.1 \text{ cm}^3$   $0.1 \text{ N H}_2\text{SO}_4$  in  $10 \text{ cm}^3$  acetonitrile solution). This does not occur for the derivative with the substituent  $\text{O}-\text{CH}_3$ . This behaviour can be interpreted by considering that water is quenched in the compounds with the  $\text{NH}_2$  substituent. This does not happen in the compound with the  $\text{O}-\text{CH}_3$  substituent.

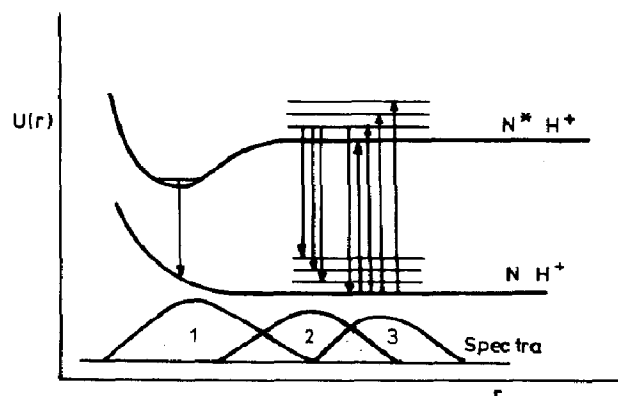


Fig. 5. Representative scheme of the absorption and fluorescence processes in acetonitrile and acidified acetonitrile (or protic media). Spectrum 1, fluorescence exciplex; spectrum 2, mirror fluorescence; spectrum 3, absorption.

This type of behaviour has been explained in a similar manner in the literature. Thus, 4-methylumbelliferone in ethanolic solution shows new fluorescence bands when acidified by hydrochloric acid [8, 9]. The new fluorescence bands are associated with protonated excited state species that are unstable in the ground state.

## 5. Conclusions

1,8-Naphthalimide derivatives with  $R_2 \equiv \text{NH}_2$  or  $\text{O}-\text{CH}_3$  form exciplexes with protons, possibly by migration of the nitrogen proton of the R chain to the excited electronic nucleus.

The formation of these exciplexes is manifested by a fluorescence shift from the mirror image position in protic media.

The exciplexes formed have constants ( $K_{\text{FM}} + K_{\text{IM}}$ ) which are much smaller than the unprotonated excited molecule. As a consequence, the lifetime increases considerably, and the same is true of the quantum yields.

Water acts as a quencher in the derivatives with  $R_2 \equiv \text{NH}_2$ ; this effect does not occur for  $R_2 \equiv \text{O}-\text{CH}_3$ .

This exciplex formation is of great interest as it may be advantageous for the use of these substances as laser dyes.

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