



Photophysical and photochemical characteristics of an azlactone dye in sol-gel matrix; a new fluorescent pH indicator

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Abstract

The photophysical and photochemical properties of an azlactone derivative, 4-(*p*-N,N-dimethylaminophenylmethylene)-2-phenyl-5-oxazolone, (DPO) in a sol-gel matrix have been examined. The fluorescence quantum yield, radiative lifetime, fluorescence lifetime, fluorescence rate constant and singlet energy values of the DPO dye were determined in a modified tetraethyl orthosilicate (TEOS) solid matrix by absorption and emission spectroscopy and obtained data were compared with the results of measurements in acetonitrile (MeCN) and tetrahydrofuran (THF) solution. The sol-gel matrix enhanced the fluorescence quantum yield ($Q_f=0.296$) and fluorescence emission lifetime ($\tau_f=0.47$ ns) compared to $Q_f=0.0027$ – 0.0025 and $\tau_f=0.02$ – 0.03 ns for solutions in MeCN and THF. The acidity constant of DPO was found to be as $pK_a=5.1$ in the transparent sol-gel matrix. The dye doped sol-gel exhibited a pH sensitivity in pH range 3.0–7.0. © 2002 Elsevier Science Ltd. All rights reserved.

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1. Introduction

Extensive studies on azlactone dyes have shown favourable photophysical and photochemical properties in the crystalline state, which has resulted in their use in semiconductor devices such as electrophotographic photoreceptors, and in non-linear optical materials [1–3]. The luminescence properties of azlactone dyes have been investigated

by several authors, and very low fluorescence efficiencies have been reported for solutions in different solvents [4–6]. The solid state fluorescence emission of the aryl derivatives of 5-oxazolones were found to be much higher in comparison to the solution state but no conclusions were drawn concerning the photophysical parameters of azlactones [3].

In recent years, the determination of pH using optical indicators has been practised intensively. Nivens and coworkers used the fluorescein isothiocyanate as a fibre-optic pH sensor in an organically modified sol-gel matrix

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[7]. Grant and Glass used a self-referencing dye, seminaphthorhodamine-1 carboxylate (SNARF-1C) in sol-gel for local blood pH measurements [8]. Qian and Xiao offered sensitive and strong fluoroionophores, 4-amino-1,8-dicyanonaphthalene derivatives, for H^+ sensing in the pH range 12–3 [9].

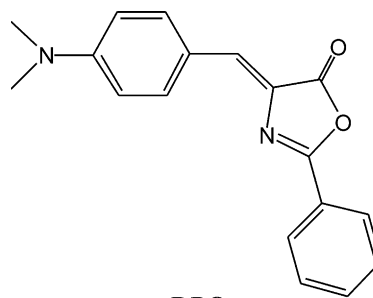
The azlactone derivative, 4-(*p*-*N,N*-dimethylaminophenylmethylene)-2-phenyl-5-oxazolone (DPO) contains several proton sensitive centres. Although a variety of fluorescent pH indicators are known DPO was chosen due to its high fluorescence quantum yield and high photostability in the solid state [3,10].

In recent years sol-gel techniques have been extensively used for incorporation of organic dyes into inorganic host matrices and this area are becoming increasingly important for optical devices. The sol gel matrix provides two different advantages for spectroscopic studies thus, it acts as a diluted and optically transparent medium like a solution, and also acts as a rigid medium like a solid phase.

It is also a valuable matrix for bio-technological and clinical applications. The chemical and mechanical stability of the matrix allows, (1) spectral measurements from UV to near IR, (2) ability of giving any desired geometric shape, (3) low temperature studies, without any solubility problems [11,12]. The sol-gel process is influenced by a number of parameters including precursor type, molar water/precursor ratio (*R*), catalyst type, pH and temperature [13,14]. The sol-gel network is formed through hydrolysis and condensation reactions. These reactions proceed as long as hydrolysible alkoxy and condensable hydroxyl groups are available [15].

In favourable cases, the immobilization of dye molecules in the solid matrix may reduce intramolecular motions and rearrangements, thus leading to enhanced photostability and fluorescence capability. Entrapment of dye molecules in sol-gel networks allows the investigation of both photophysical and photochemical properties and sensor applications. pH dependent changes in the absorption and emission spectra of certain fluorescent indicators in sol-gel matrices can be followed by optical detection.

In the present work, the photophysical and photochemical properties of DPO in acid catalyzed and acid catalyzed/buffer modified sol-gel matrices, are reported. These pH sensor properties and photophysical parameters are compared to those obtained for DPO in acetonitrile and tetrahydrofuran solutions.



DPO

2. Experimental

2.1. Materials

DPO, 4-(*p*-*N,N*-dimethylaminophenylmethylene)-2-phenyl-5-oxazolone, was synthesized and purified as described previously [2]. The sol-gel components; tetraethyl orthosilicate (tetraethoxysilane, TEOS), and tetramethyl orthosilicate (tetramethoxysilane, TMOS), were supplied from Merck and Fluka, respectively. Analytical grade concentrated hydrochloric acid was from Merck. The acidic test solutions were prepared in the buffering agent of *N,N*-bis(2-hydroxyethyl)-2-amino-ethanesulfonic acid (BES), from Sigma. All of the solutions and buffers were prepared using high quality pure water of Elga Maxima. The surfactant additive triton X-100 (polyethylene glycol *t*-octylphenyl ether) was obtained from Merck. Regeneration of the sensor was accomplished with titrisol buffer from Merck. Absolute ethanol or methanol used throughout the studies, were used as received. The reference standard for fluorescence quantum yield measurement of the dye was *n*-dodecylperylene diimide ($Q_f = 1$ in $CHCl_3$) [16].

2.2. Sol-gel matrix and film preparation process

DPO doped silica gel glasses were prepared by hydrolyzing TEOS/TMOS. 1 millilitre of 2 mM

DPO (in THF) was mixed with 1.5 ml of absolute ethanol/methanol in a glass vial and sonicated for 1 minute. Afterwards 1 ml of TEOS or TMOS was added into the dye solution and sonicated for 5 min. This was then followed by the addition of 1 ml of acidic water (pH=2 via addition of HCl) and 20 μ l of triton X-100 in order to improve the homogeneity of the silica sol-gel and to give a crack-free monolith. In all cases the solutions were aged at room temperature in closed glass vials. Since there was no visual difference between both the absorption and emission spectra obtained from the TMOS and TEOS precursors, the non-toxic one, TEOS, is used throughout the studies.

Glass slides (11 \times 40 \times 1 mm) were used as solid support onto which the sol-gel was cast by manual dip-coating technique. Prior to casting, the glass surface was activated by treatment with concentrated HNO₃ for 24 h, washed with distilled water, and then ethanol or methanol. After evaporation of the solvents in a dessicator, the glass slides were fixed diagonally in a quartz sample cuvette. The advantage of this kind of a placement was to improve the reproducibility of the measurements. Film thickness of the glass slides were measured using Tencor Alpha Step 500 Prophylometer and found as 2.096 μ m. This result is an average of eight measurements and exhibits a standard deviation of ± 0.0086 .

2.3. Spectroscopic measurements

The absorption spectra of sol-gel glass slides with approximately 2 μ m thicknesses were measured using a Jasco V-530 UV–vis spectrophotometer. The fluorescence emission spectra were recorded using a PTI-QM1 fluorescence spectrophotometer. pH measurements were performed with a pH-meter Jenway 3040 Ion-Analyser calibrated with Merck pH standards of pH 7.00 (titrisol buffer) and pH 4 at 20 °C. Fluorescence quantum yield of the dye were measured by reference to the fluorescence emission of *n*-dodecylperylene-3,4,9,10-tetracarboxylic diimide in sol-gel matrix using an excitation wavelength of 492 nm.

3. Results and discussion

3.1. Matrix modification and effect of surfactant

An important consideration in the preparation of DPO doped films is the pH of the reaction medium. The initial hydrolysis of the sol-gel process requires catalysis at either acidic or basic pH, and, direct addition of DPO into the acid catalyzed sol-gel composition may result in a less sensitive sensor glass, as the emission signal of DPO dye decreases in acidic solutions. Increasing the pH of the sol-gel composition just prior to the addition of the dye by using a pH buffer enhances the signal intensity. Before the addition of the acid sensitive dye, a sufficient quantity of concentrated phosphate buffer (250 μ l, 0.14 M, pH=6) was added, and the pH of the sol was increased. This buffer-modified sol-gel composition was used for coating [17].

Since the surfactant free samples showed cracking, Triton X-100 was added to improve the homogeneity. According to Brinker and Scherer, addition of surfactant provides a higher level of homogeneity. Polymerization in the presence of a surfactant increases the molecular mass and decreases the inner tensions in the glass that occurs during condensation and polycondensation, as a result the cracks do not form [18].

Physical examination of the coating under a low power microscope showed an intact surface with no visible cracks.

3.2. Photophysical characteristics of DPO

Absorption and fluorescence emission of DPO in the crystalline state [3], in MeCN, THF, and in plasticized PVC films have been reported earlier [10]. In this work, the absorption and emission based photophysical parameters of DPO in the sol-gel matrix have been extracted from spectral data and are summarised in Table 1. The radiative lifetimes τ_o , were calculated by the formula: $\tau_o = 3.5 \times 10^8 / \nu_{\max}^2 \cdot \epsilon_{\max} \cdot \Delta\nu_{1/2}$, where ν_{\max} is the wavenumber in cm^{-1} , ϵ_{\max} is the molar extinction coefficient at the selected absorption wavelength, and $\Delta\nu_{1/2}$ is the half width of the selected absorption in wavenumber units of cm^{-1} .

Table 1

UV–vis spectroscopy data (λ : nm, and ϵ : $\text{lmol}^{-1}\text{cm}^{-1}$), Stokes' shifts ($\Delta\gamma$: cm^{-1}), fluorescence quantum yield, Q_f , radiative lifetime, τ_o (ns), fluorescence lifetime τ_f (ns), fluorescence rate constant, k_f (10^9 s^{-1}), and singlet energy, E_s (kcal/mol) of DPO in acetonitrile (MeCN) solution, in tetrahydrofuran (THF) solution and in the sol-gel matrix

Solvent	$\lambda_{\text{max}}^{\text{abs}}$	ϵ_{max}	λ_{max}	$\Delta\gamma$	Q_f	τ_o	τ_f	k_f	E_s
MeCN [10]	465	51 000	527	3190	0.0027	7.2	0.02	52.6	61.5
THF	463	21 000	525	2623	0.0025	11.4	0.03	34.5	61.8
Sol-gel mat.	492	208 000	537	1704	0.296	1.6	0.47	2.1	58.4

The sol-gel glass slides were stored in a desiccator and were reusable. The absorption maximum, $\lambda_{\text{max}}^{\text{abs}}$, of DPO in the sol-gel matrix is 27 nm red shifted with respect to MeCN solution, and 29 nm red shifted relative to THF (Fig. 1). As a result, the excited singlet state energy, E_s of DPO was found to be 58.4 kcal/mol in the sol-gel matrix and the singlet transition of DPO is 3.1 and 3.4 kcal/mol lower than in MeCN and THF solutions, respectively, which may be related to enhanced polar interactions between the DPO molecule and the sol-gel structure. As stated by Wolfbeis, depending on the nature of interaction between the dye and its microenvironment, the dye may undergo not only spectral shifts, but also other polarity dependent changes may occur (i.e. molar absorptivity, fluorescence quantum yield differences and others) [18]. The fluorescence emission band of DPO in sol-gel is shifted 9 nm to shorter wavelengths in MeCN and 12 nm to longer wavelengths in THF. The Stokes' shift of DPO in the sol gel matrix lies between the two corresponding values in the organic solvents. On the other hand, the calculated fluorescence quantum yield and calculated radiative and fluorescence lifetimes of DPO in sol-gel, are higher than corresponding value in organic solvents [found $Q_f=0.296$, $\tau_o=1.6$ ns, and $\tau_f=0.47$ ns, (Table 1)]. The 100-fold higher Q_f value may be attributed to restricted mobility of DPO in sol-gel cages.

3.3. Photostability of DPO

The photostability of DPO dye in the sol-gel matrix and in solutions of MeCN and THF was determined with a steady-state spectrofluorimeter in time based mode. The data were acquired at 537

nm in sol-gel matrix, 525 nm in THF and 527 nm in MeCN solutions. These wavelength values correspond to the emission wavelength maximum of DPO in the three different media using an excitation wavelength of 480 nm. The data collected after 1 h monitoring is shown in Fig. 2. According to our earlier experimental and theoretical evaluations [2,3] low fluorescence quantum yields and photoinstability of azlactones in solution are not caused by efficient intersystem crossing but rather from solvation effects, which results in increased vibrational flexing of the molecule. The excellent photostability in sol-gel and the lower photostability in fluid solution can be seen in Fig. 2.

3.4. DPO, as a pH indicator

The DPO molecule can be protonated in acidic media and the process of protonation is irreversible due to ring opening in solution. In the sol-gel matrix the protonation–deprotonation equilibrium is completely reversible. Detection of fluorescence emission signals allows us to develop an optode pH sensor for the pH range of 3.0–7.0.

In this context, the glass slides were coated with the dye doped sol-gel and exposed to different concentrations of hydrochloric acid solution. pH induced fluorescence emission spectra between pH 3.0 and 7.0 were recorded for acid catalyzed and buffer modified–acid catalyzed matrices. In both cases the proton sensitive composition responds to different concentrations of HCl solutions (in BES buffer) by a decrease in fluorescence intensity that can be used as the analytical signal. As described earlier, for estimation of the relationship between the pH of the matrix and the response of the dye, both the acid catalyzed and

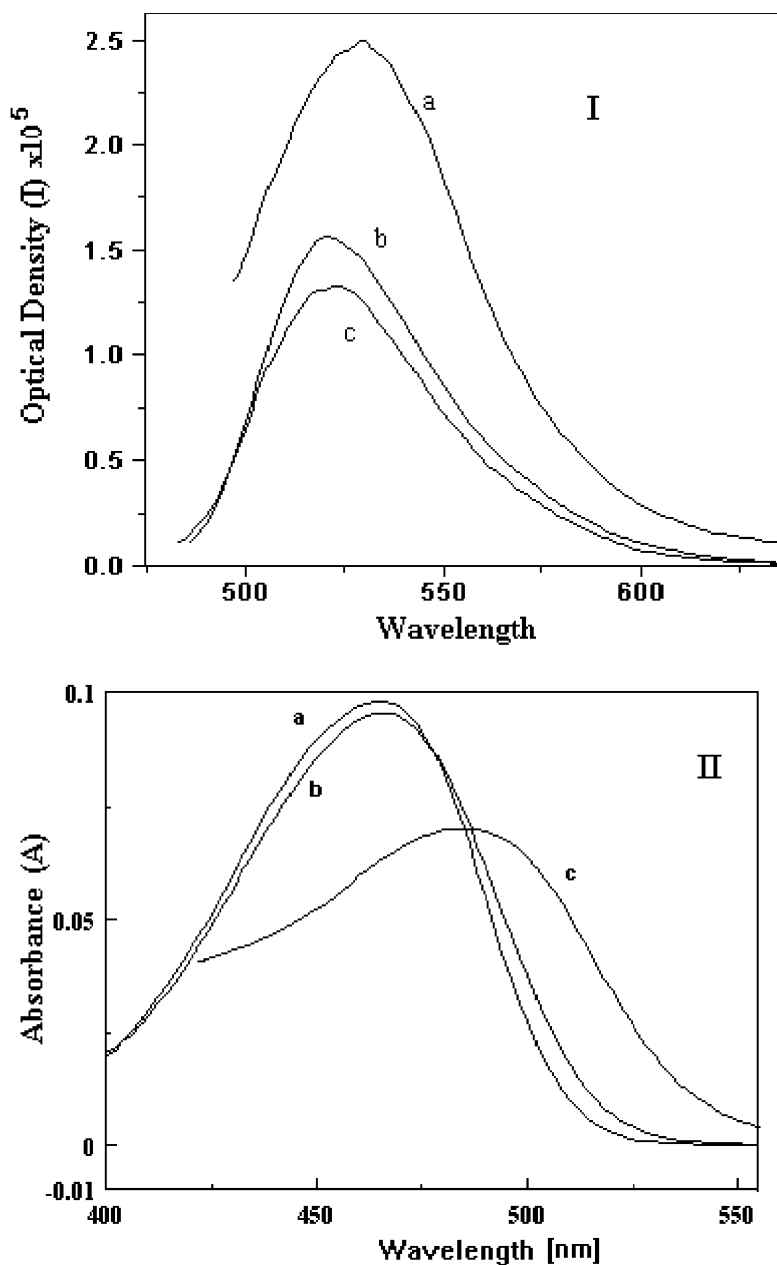


Fig. 1. (I) Emission spectra of DPO (a) in sol-gel ($\lambda_{\text{max}} = 537$ nm) (b) in THF ($\lambda_{\text{max}} = 525$ nm) and (c) in MeCN ($\lambda_{\text{max}}^{\text{em}} = 527$ nm). (II) Absorption spectra (a) in THF (b) in MeCN (c) in sol-gel matrix.

acid catalyzed-buffer modified matrices can be compared [10].

The fluorescence emission intensity of the dye in sol-gel revealed significant differences as a func-

tion of the pH of the matrix (Fig. 3). When the DPO dye was doped into the conventional acid-catalyzed matrix, during pH induced studies, a 9 nm of blue-shift was observed [Fig. 3(I)]. In contrast, in

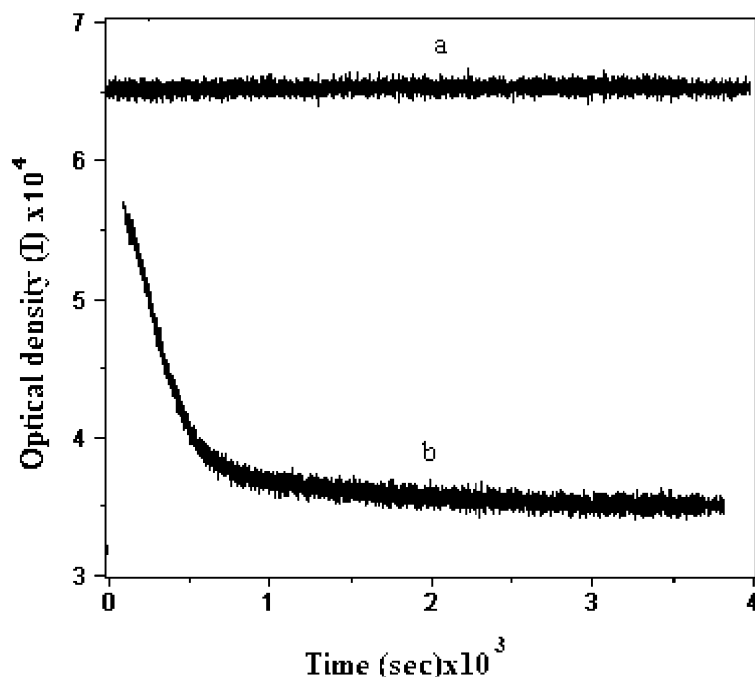


Fig. 2. The photostability test of azlactone dye (a) in sol-gel matrix (b) in solvent, after 1 h of monitoring.

the case of the acid catalyzed buffer-modified matrix, no spectral shift was observed. It was also found that a more pronounced relative emission intensity change is obtained in the buffer modified-acid catalyzed sol gel matrix with respect to the simply acid catalyzed matrix. Whilst the acid catalyzed matrix exhibits 20% relative emission intensity change, the buffer modified matrix exhibited a 64% change.

3.5. pK_a values

The pH-induced emission spectra of DPO are shown in Fig. 3(II). A plot of the normalized fluorescence intensity versus pH, after immobilization in the sol-gel matrix, yields an S shape curve with one inflection point (Fig. 4). Normalized emission parameters (I/I_{\max}) are calculated by division of measured fluorescence intensity at neutral pH by I_{\max} ($\text{pH} \cong 7$). Data in Fig. 4 are the average of five replicate measurements each with fresh sensor glass and the relative standard deviation (RSD) is calculated as 3–4%. The

recorded standard deviation may be due to the manual dip-coating technique and can be attributed to the difficulties of reproducibility of sensor glass preparation. The pK_a value was calculated to be 5.2 from the normalized fluorescence intensity plot. This result indicates that DPO may be an effective optical pH sensor in the pH 3.0–7.0 range.

3.6. Reproducibility and reversibility of the sensor

Fig. 4 outlines the pH induced fluorescence intensity versus time. (0.02 M BES buffered HCl and NaOH solutions were used throughout).

The reproducibility and reversibility of the sensor were tested by exposing the glass slides to the highest concentrations of HCl until $\text{pH} = 3$. After the first cycle, there was a significant drift (about 10%) in sensor response, which can be concluded as conditioning step. Once encapsulated and conditioned, leaching of the DPO out of the sol-gel matrix is very low, which is an advantage of the matrix. The following second and third cycles did not result in

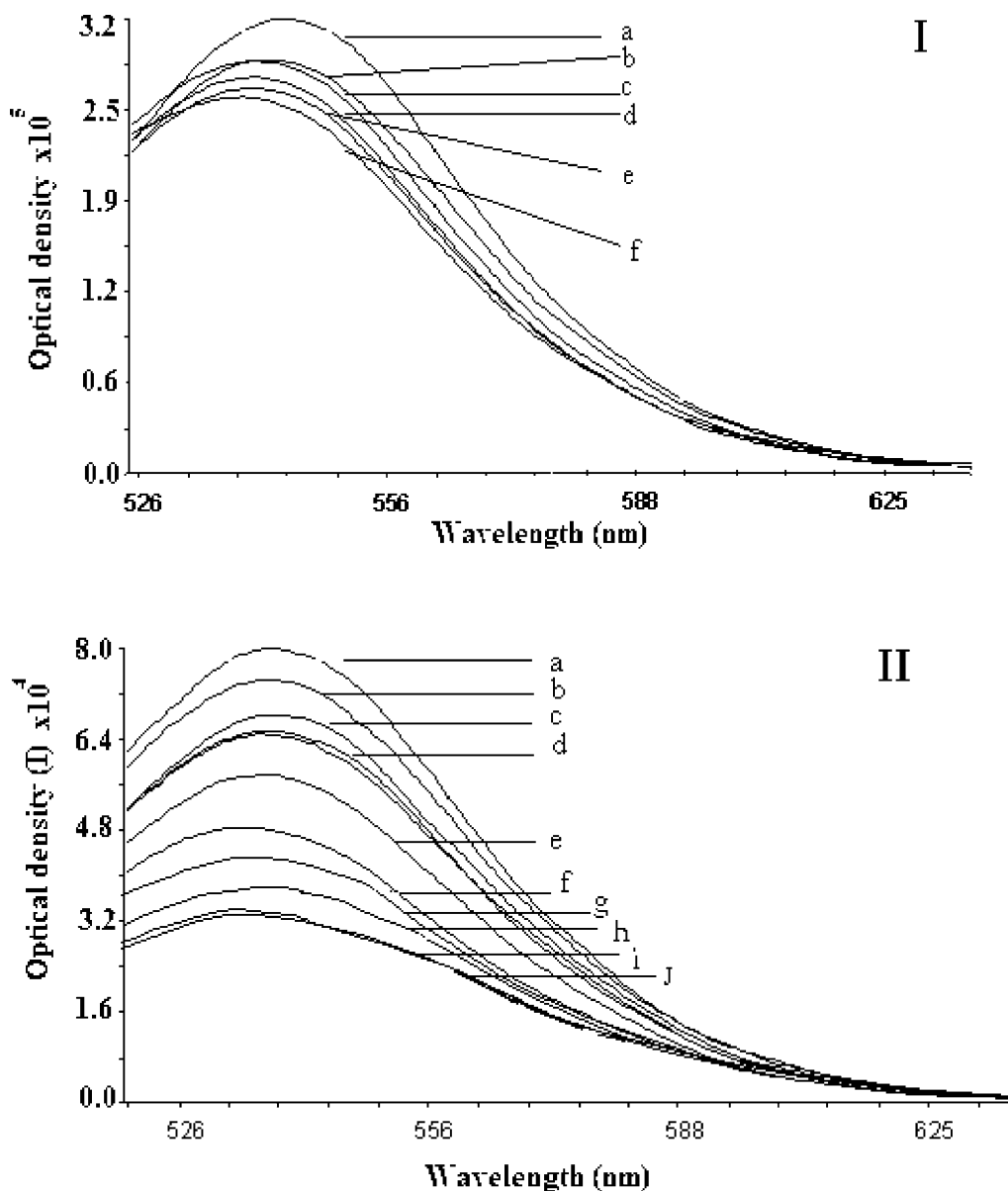


Fig. 3. pH induced emission spectra of azlactone dye in acid catalyzed (I) and acid catalyzed-buffer modified (II) matrices, at pHs (a) 7.0, (b) 6.8, (c) 6.2, (d) 5.8, (e) 5.4, (f) 4.8, (g) 4.2, (h) 3.5, (i) 3.0, (j) 2.8.

any further large drifts (Fig. 5). The reproducibility of the optical responses was assessed by repeatedly introducing a sample of pH=3.0 and a titrisol buffer at pH=7.0. Between second and fifth cycles, the level of reproducibility achieved corresponded to a RSD of 1.4%.

The sensor was fully reversible in the pH range 3.0–7.0. It was also observed that the colour of the glass slide was yellow at pH = 7.0, but gradually changed to orange as the pH reached to 3.0, and a reverse colour change was observed on returning to pH 7.0. The response time (τ_{90}) was

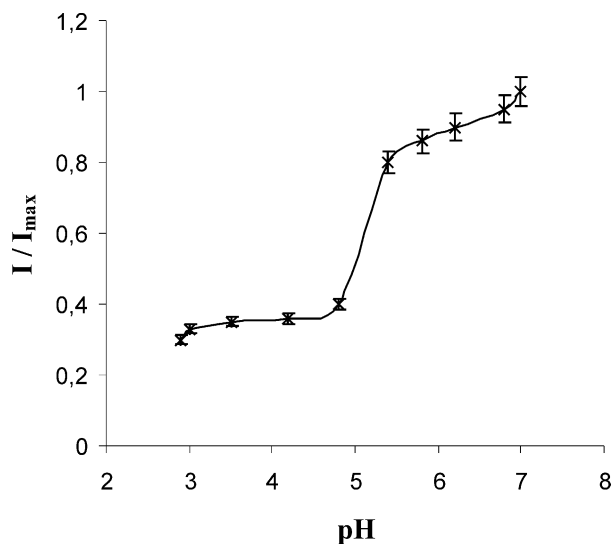


Fig. 4. Normalized fluorescence intensity vs. pH in buffer modified sol-gel matrix. The error bars represent the standard deviation ($n = 5$).

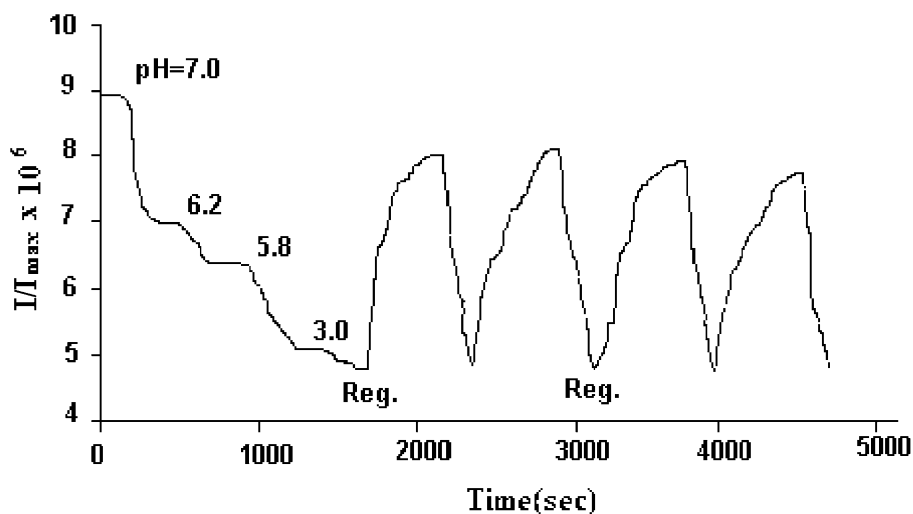


Fig. 5. The pH induced fluorescence intensity vs. time to evaluate the reproducibility and reversibility of the sensor. For regeneration the titrisol buffer and for pH induced studies 0.02 M BES buffered HCl and NaOH solutions were used.

measured as 4.5 min under batch conditions (Fig. 5).

The problem in terms of reversibility was slow deprotonation. The binding of a proton was fast but its release was slow. The reagent phase could be regenerated by a $\text{H}_2\text{PO}_4^-/\text{HPO}_4^{2-}$ buffer system with a pH of 7 ± 0.02 . The regeneration time under batch conditions was about 40 min.

Sensor stability was tested on three consecutive months at different acid concentrations in BES buffer and was found to be satisfactory. Fig. 6 shows the fluorescence intensity ratio vs. pH for the same sensor for three consecutive months of measurements. The data displayed an average correlation coefficient of 0.992.

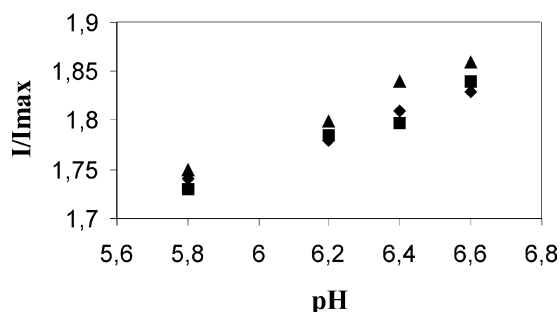


Fig. 6. Comparison of a single sensor response to pH in phosphate buffer obtained on three consecutive months, month 1 (▲), month 2 (■), month 3 (◆).

4. Conclusion

The fluorescent azlactone dye, DPO has a reasonable fluorescence quantum yield in the sol-gel matrix and its long wavelength excitation maximum enables its usage with a blue light emitting diode (LED) and optical fibres. The sensor has a reproducible pH response in the pH range 3.0–7.0, which covers the clinically important region. It is known that acid catalyzed sol-gel matrices are widely preferred to base-catalyzed ones, due to shorter hydrolysis and longer gelation times. DPO, introduced as a pH sensor, becomes less functional in an acidic matrix, and therefore, the pH of the sol-gel matrix needed to be increased by the use of a suitable buffer. By using such a buffer short hydrolysis times were achieved and the negative effect of strongly acidic media, results from an acid-catalyzed sol-gel matrix, on the pH sensitive dye was avoided. It was found that the microenvironment has a significant effect on the photophysical and photochemical parameters of the dye. High fluorescence quantum yields and good photostability in the solid state are attributed to the rigidity of the azlactone molecule in solid matrix.

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