Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Photochemistry and Photobiology A: Chemistry 169 (2005) 221–228
Journal of Photochemistry and Photobiology A:Chemistry
www.elsevier.com/locate/jphotochem

Fluorescence studies of the sol–gel transition using aminopyrene

Ewa Miller*, Stanisław Wysocki, Donata Jóźwik

Institute of General Food Chemistry, Technical University of Łódź, ul. Stefanowskiego 4/10, 90-924 Łódź, Poland

Received 15 June 2004; accepted 8 July 2004
Available online 12 August 2004

Abstract

The process of tetraethylorthosilicate gelation in the acidic medium was monitored by a fluorescence probe of aminopyrene using its concentration and excitation wavelength. Steady-state and time-resolution fluorescence measurements were recorded on the consecutive days of gelation. The results were compared with experimental data for model water solutions of aminopyrene at different pH. On this basis, the character of probe molecule interaction with a changing microenvironment was determined. The state of equilibrium of two forms of aminopyrene: $APH^+ \leftrightarrow AP + H^+$ on the consecutive days of sol–gel transition was discussed. It was a measure of changes that took place during gel net formation.

Keywords: Sol–gel; Silane; Fluorescence; Aminopyrene

1. Introduction

The application of silane gels as a carrier for an organic probe in the construction of optical sensors and chemical biosensors is a very attractive solution owing to mild conditions of sol preparation, easy processing, and such gel properties as transparency, hardness and a composition similar quartz [1–3]. As a result of gelation that consists of hydrolysis and condensation of silane monomers, organic molecules, e.g. biologically important compounds, enzymes and fluorescence probes can be immobilized in the gel without destroying them [4–7]. From the point of view of applications it is also important that compounds immobilized in the gel be not eluted by a solvent during the sensor operation, which basically affects stability of this operation.

During the sol–gel transition, significant changes occur in the polarity, microacidity and viscosity in the fluorophore microenvironment, which result from changes in the closest molecule environment induced by the transition from sol to gel, i.e. during the formation of a gel net [8–11]. The probes, whose fluorescence emission spectra depend on the molecule environment, include among the others, pyrene and its derivatives such as carboxypyrene and aminopyrene (AP) [12–14]. Thomas and co-workers used this fluorophore to identify the type of hydroxyl groups on the surface of silicon oxide [13] or aluminum oxide [15].

Owing to its structure and the presence of $-NH_2$ amino group, aminopyrene is sensitive to changes in the microenvironment that can lead to protonation of $-NH_3^+$ group according to the formula

$$\underset{\text{emission typical of pyrene}}{APH^+} \leftrightarrow \underset{\text{emission typical of aminopyrene}}{AP} + H^+ \quad (1)$$

The fluorescence spectrum typical of aminopyrene, with a maximum at 440 nm, formed as a result of mixing the $\pi \rightarrow \pi^*$ transitions of benzene ring and $n \rightarrow \pi^*$ of nitrogen electrons in the amino group changes when the interaction of a free pair of electrons in nitrogen and π-electrons of pyrene rings for APH^+ are blocked [13]. As a result, for the protonated form of aminopyrene the emission spectrum typical of pyrene is observed.

In this paper an attempt was made to apply the photochemical properties of AP in monitoring of the sol–gel transition.

* Corresponding author. Tel.: +48 42 6313426; fax: +48 42 6362860.
E-mail address: emiller@snack.p.lodz.pl (E. Miller).

1010-6030/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.jphotochem.2004.07.007

2. Experimental

Tetraethylorthosilicate (TEOS) (Sigma), 1-aminopyrene (Aldrich), spectrally pure anhydrous ethyl alcohol (P.O.Ch., Gliwice, Poland) and deionized water were used in the measurements.

A sol of tetraethylorthosilicate (TEOS), ethanol and water in the molar ratio 1:6:6 was prepared. 1-Aminopyrene was introduced to the sol in the form of ethanol solution at concentrations 5×10^{-4}, 5×10^{-6} and 5×10^{-7} M. After 2 h mixing, a sol of pH = 4.5 was obtained. The pH of sol was adjusted by adding properly diluted HCl. The sol gelated for 4 days.

Steady-state measurements of excitation and fluorescence emission of 1-aminopyrene placed in the input silane sol, and on the consecutive days of gelation were made using a FLUOROMAX-2 spectrofluorimeter (Jobin Yvon).

Time-correlated measurements were conducted using a single-photon-counting system from Edinburgh Analytical Instruments (FL 900CDT).

3. Results and discussion

3.1. Steady-state fluorescence spectra

Three samples of silane sol of the composition described above, with different concentrations of aminopyrene were prepared. Fluorescence emission spectra of the probe were recorded on the consecutive days of gelation. On the basis of a prior research pH = 4.5 was assumed for sol, which was a precondition of gel formation after 4 days [8].

Fig. 1 shows fluorescence emission spectra of aminopyrene at the concentration 10^{-4} M on the 1st day of gelation in the sol (Fig. 1A) and on the 9th day in the gel already (Fig. 1B), at different excitation wavelengths. With an increase of the excitation wavelength the intensity of fluorescence emission increases both in the sol and in the gel. Using such a probe concentration, a predominant inert form of AP with a maximum emission at the wavelength 430 nm is observed. The intensity of fluorescence emission for a given wavelength decreases during the gelation process. The character of AP spectrum with a maximum emission λ = 430 nm in the sol confirms the nature of the AP spectrum in the water-ethanol environment [13].

Sols containing aminopyrene of lower probe concentrations: 5×10^{-6} and 5×10^{-7} M, were also prepared. Using the concentration of AP 5×10^{-6} in the sol, the fluorescence spectra were recorded at λ_{ex} = 313 nm for the sol–gel transition and a band ranging from 360 to 400 nm was observed. The band intensity increased during the gelation process (Fig. 2). On every day of gelation the fluorescence emission spectra of AP as a function of the excitation wavelength were also recorded (Fig. 3). Fig. 3A illustrates consecutive experimental data on the 1st day in the sol, while Fig. 3B shows the data on the day of sample gelation, i.e. on the 4th day. During the sol–gel transition the inert form of AP with a maximum emission at λ = 430 nm dominates in the system and, as mentioned previously, an additional band in the range 360–400 nm appears. It decreases with the transition of the excitation wavelength to longer wavelengths and practically decays at the excitation wavelength of 340 nm. As known from literature and previous research on pyrene concerning investigation of sols and silane gels by fluorescence methods, a subtle spectrum in the short-wave region is characteristic of this probe [9,16,17]. When aminopyrene is applied as a fluorophore (Eq. (1)) such a character of the spectrum is typical of its protonated form [13].

For comparison, the fluorescence emission and excitation spectra of aminopyrene at the concentration 10^{-7} M were recorded as a function of pH of the water probe solution

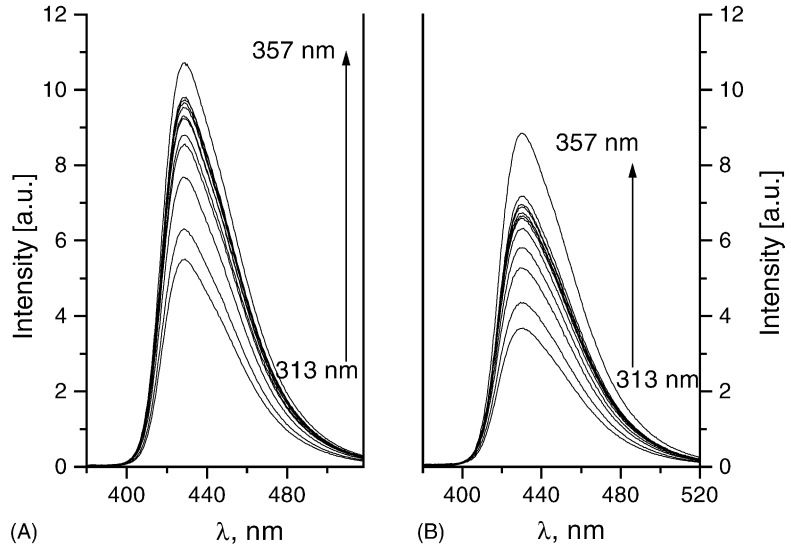

Fig. 1. Dependence of fluorescence emission of aminopyrene on the excitation wavelength for selected days of the gelation process: (A) 1st day (sol), (B) 9th day (gel); $c_{AP} = 10^{-4}$ M.

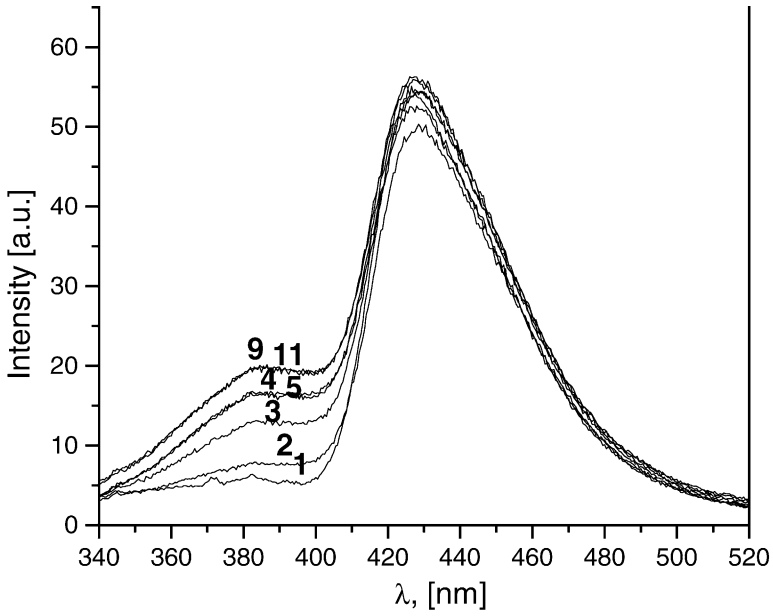

Fig. 2. Dependence of AP fluorescence intensity on the consecutive days of gelation $c_{AP} = 5 \times 10^{-6}$ M, $\lambda_{ex} = 313$ nm.

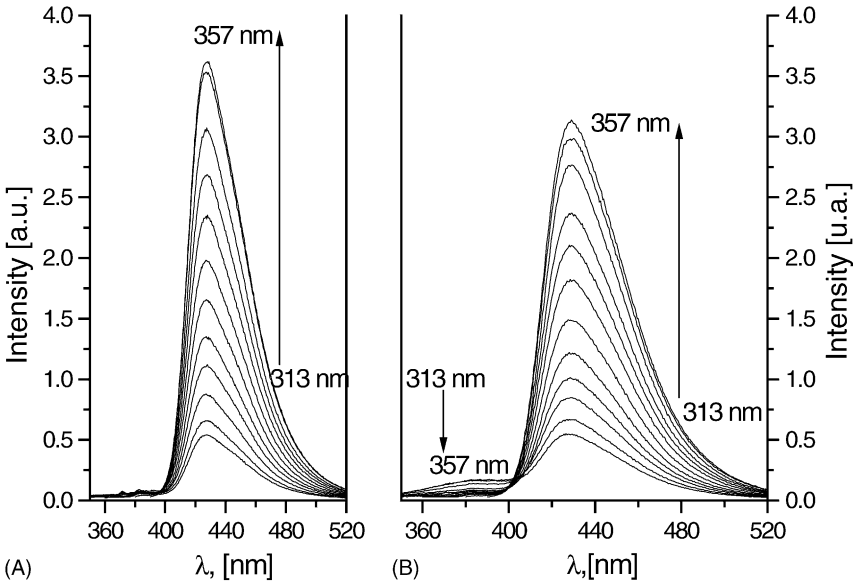

Fig. 3. Intensity of fluorescence emission for aminopyrene as a function of the excitation wavelength: (A) on the 1st day of gelation (sol), (B) on the 4th day of gelation (gel); $c_{AP} = 5 \times 10^{-6}$ M.

(Fig. 4). On this basis the value of pK_a was determined which equalled 4.8 and the concentration of APH^+ protonated form was estimated depending on the pH of water solutions (Table 1). Additionally, it was observed that the emission spectrum was typical of an inert form of aminopyrene in the whole range of applied pH (Fig. 4A). The excitation spectra for pH 2.0–3.5 diverged from the spectra for other pH values and resembled the pyrene spectrum (Fig. 4B). This shows that the APH^+ form can be observed only by analyzing the excitation spectrum of the probe in the acidic environment, at pH <4, when the concentration of APH^+, $c_{APH^+} > 5.8 \times 10^{-8}$ M (Table 1). At pH exceeding 4, in the excitation state of

Table 1
Concentration of APH^+ (c_{APH^+}) as a function of pH of the solution $c_{AP} = 10^{-7}$ M

pH	1.9	2.8	3.2	3.4	4.7	5.1	5.7	6.4	8.3	9.5	10.7	11.9
c_{APH^+} (M)	9.98×10^{-8}	9.91×10^{-8}	9.80×10^{-8}	9.62×10^{-8}	5.85×10^{-8}	3.87×10^{-8}	1.37×10^{-8}	3.16×10^{-9}	3.55×10^{-11}	2.24×10^{-12}	1.58×10^{-13}	8.91×10^{-14}

Calculations were made using the relation: $c_{APH^+} = c_{AP}/1 + 10^{(pH-pK)}$.

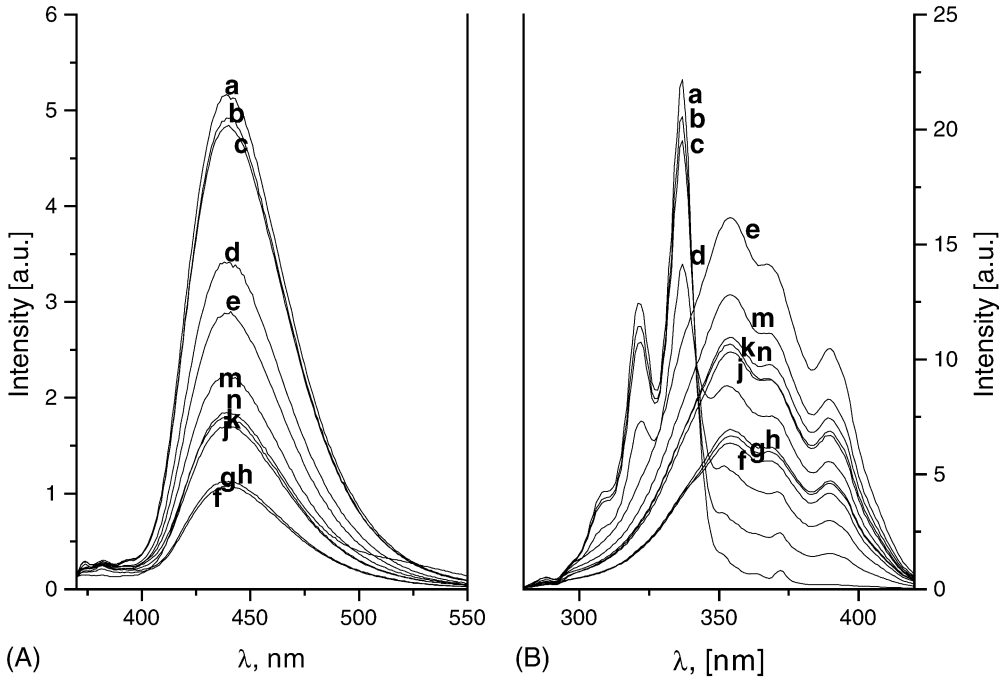

Fig. 4. (A) Fluorescence emission and (B) excitation spectra for aminopyrene as a function of pH: (a) pH 2.0, (b) pH 2.8, (c) pH 3.2, (d) pH 3.5, (e) pH 4.7, (f) pH 5.1, (g) pH 5.7, (h) pH 6.4, (j) pH 8.3, (k) pH 9.5, (m) pH 10.7, pH 11.9; $c_{AP} = 5 \times 10^{-6}$ M.

the molecule, dissociation prevails and the APH^+ form is not visible either in the emission or excitation spectra. Thomas and co-workers [13,15] observed a similar effect when analyzing absorbance and emission spectra of aminopyrene in water solutions at low pH.

For the AP of the concentration 5×10^{-6} M in the excitation spectra recorded during the process of gelation, no APH^+ form was found (Fig. 5A). The excitation spectra for aminopyrene, at probe concentration 10^{-7} M (Fig. 5B), recorded on the consecutive days of gelation, have the form typical of pyrene. This effect might indicate an increasing concentration of the protonated form of aminopyrene observed during the gelation as a result of the interaction between the molecule and proton coming from the microenvironment. As a result, the interaction of a free pair of nitrogen electrons in the amino group with an aromatic ring of the molecule is blocked and a spectrum similar to that of pyrene is observed during the fluorescence emission [13]. It is known

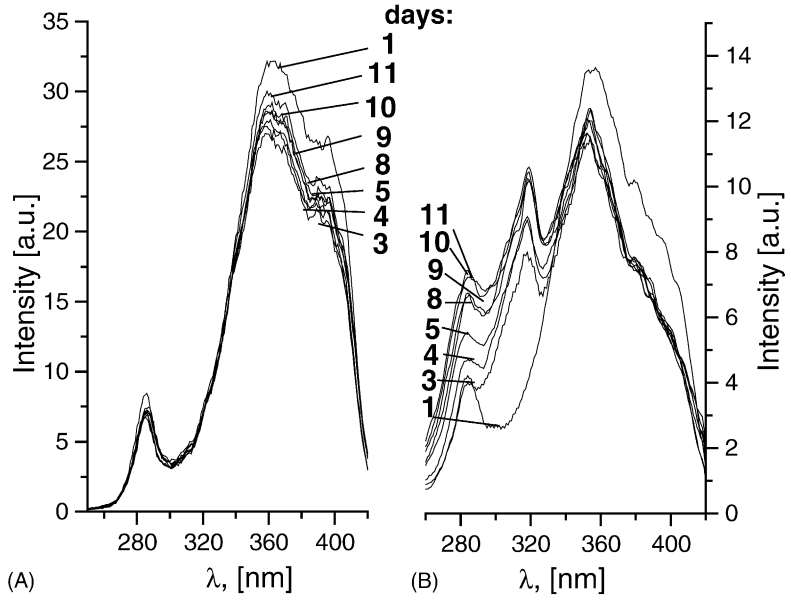

Fig. 5. Fluorescence excitation spectra of AP on the consecutive days of gelation; (A) $c_{AP} = 5 \times 10^{-6}$ M, (B) $c_{AP} = 10^{-7}$ M; $\lambda_{em} = 430$ nm.

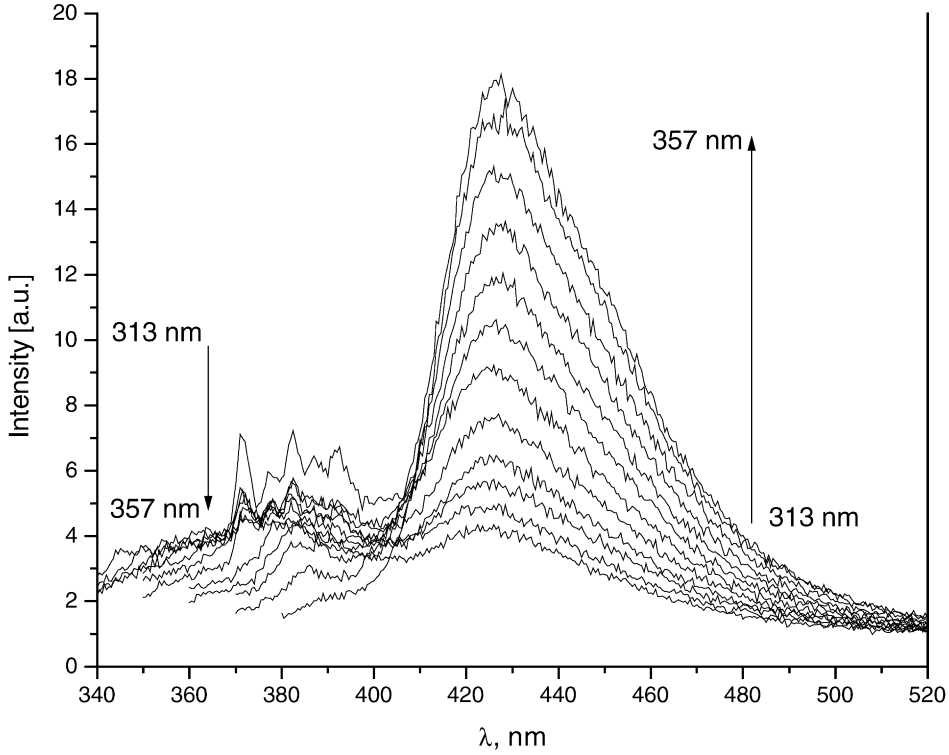

Fig. 6. Dependence of AP fluorescence emission on the 1st day of gelation (sol); $c_{AP} = 10^{-7}$M.

that hydroxyl groups are formed at the first stage of gelation, as a result of silane monomer hydrolysis. Most of these groups participate then in the reaction of condensation and gel formation, while some small part remains unbound in the gel net [18–20]. It is suggested that aminopyrene molecules interact with the proton of unbound hydroxyl groups. Therefore, the effect is discernible only at such probe concentration that is comparable to the concentration of hydroxyl groups unbound in the gel net and it is enhanced in the formed gel.

To confirm the tests, the fluorescence emission spectra of aminopyrene at the concentration 10^{-7} M were recorded on the 1st, 4th and 9th day of the gelation process (Figs. 6 and 7). On the 1st day, the spectrum with a maximum emission at the wavelength 430 nm and another one, with lower intensity in the range from 360 to 400 nm, dominate in the sol (Fig. 6). On the 4th day of the process, i.e. on the day of gelation (Fig. 7A), a distinct band appears at 360–400 nm, which decreases with an increase of the excitation wavelength, at the

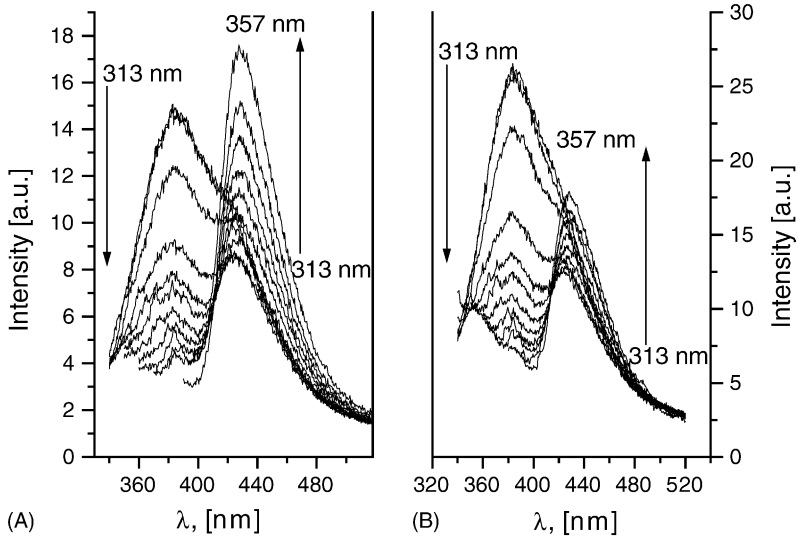

Fig. 7. Dependence of AP fluorescence emission on the 4th and 9th day of gelation (gel); $c_{AP} = 10^{-7}$ M.

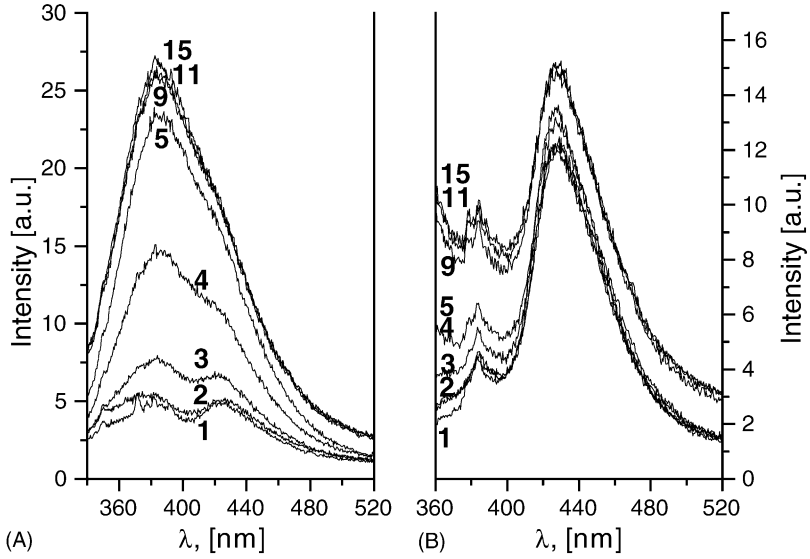

Fig. 8. Intensity of AP fluorescence on the consecutive days of gelation for: (A) λ_{ex} = 313 nm, (B) λ_{ex} = 345 nm.

cost of formation of a less energetic band with a maximum at 430 nm. From experimental data shown in Fig. 7B it follows that in the gelated sample after 5 days of storage at the temperature 20 °C the emission in the range from 360 to 400 nm doubled, while the spectrum intensity at maximum 430 nm did not change.

Additionally, basing on the character of fluorescence spectra of the probe recorded during the gelation, a conclusion may be drawn that part of –OH are the geminal groups. These groups interact with each other and form hydrogen bonds. Suitable centers for water molecule adsorption are formed in this way [12]. From studies carried out by Thomas and co-workers it follows that aminopyrene is adsorbed on silica surface near these centers just in the protonated form [13,15].

Changes in the fluorescence intensity observed on the consecutive days of gelation depend significantly on the excitation wavelength (Fig. 8). At the excitation wavelength equal to 313 nm, during the gelation process the band intensity grows significantly in the range of 360–400 nm (Fig. 8A), which is responsible for the form of APH^+ probe. On the basis of the intensity of the probe fluorescence emission at λ_{em} = 385 and λ_{em} = 430 nm, corresponding to the protonated and non-protonated form of aminopyrene, the local pH of the probe microenvironment was estimated, and next the APH^+ concentration was determined on the consecutive days of gelation (Table 2). As expected, a slight decrease of pH of the sol was reported on the consecutive days until the day of gelation and an over 20% increase of the APH^+ concentration was observed in the same time. After several days, equilibrium was established in the gel on the level of 6.4×10^{-8} M of APH^+ content. For the excitation wavelength of 345 nm on the consecutive days of gelation the band intensity assumed smaller values, and a predominant probe form was AP with a maximum at 430 nm (Fig. 8B).

On the basis of emission spectra for the probe at the concentrations 5×10^{-6} and 5×10^{-7} M, recorded on the consecutive days of the gelation process, the I_{385}/I_{430} ratio, i.e. the ratio of a maximum fluorescence emission intensity of the protonated and inert form of aminopyrene was determined (Fig. 9A and B). This quotient assumed values higher for shorter excitation wavelengths and higher when a probe of lower concentration was used. I_{385}/I_{430} increased linearly until the day of the sample gelation, which confirmed a growing contribution of APH^+ with an increase of the system viscosity and gel net formation. I_{385}/I_{430} in the gel took on a constant value, which showed that equilibrium was set up between the AP and APH^+ forms. This value for λ_{ex} = 313 nm and aminopyrene concentration 10^{-7} M is equal to 1.72. When aminopyrene concentration is 5×10^{-6} M the value of I_{385}/I_{430} reaches 0.35.

3.2. Lifetime measurements

Time-resolution measurements were also carried out for the process of TEOS gelation at pH of the starting sol be-

Table 2
pH i c_{APH^+} on the consecutive days of gelation $c_{AP} = 10^{-7}$ M

Days of gelation	State	pH	c_{APH^+} (M)
1	Sol	4.86	4.94×10^{-8}
2	Sol	4.81	5.23×10^{-8}
3	Sol	4.71	5.79×10^{-8}
4	Sol–gel	4.66	6.08×10^{-8}
5	Gel	4.61	6.35×10^{-8}
9	Gel	4.61	6.35×10^{-8}
11	Gel	4.60	6.40×10^{-8}
15	Gel	4.61	6.35×10^{-8}

Calculations were made using the relation: pH = log I_{385}/I_{430} + pK_a, where I_{385}: intensity of the probe fluorescence emission at λ = 385 nm, I_{430}: intensity of the probe fluorescence emission at λ = 430 nm.

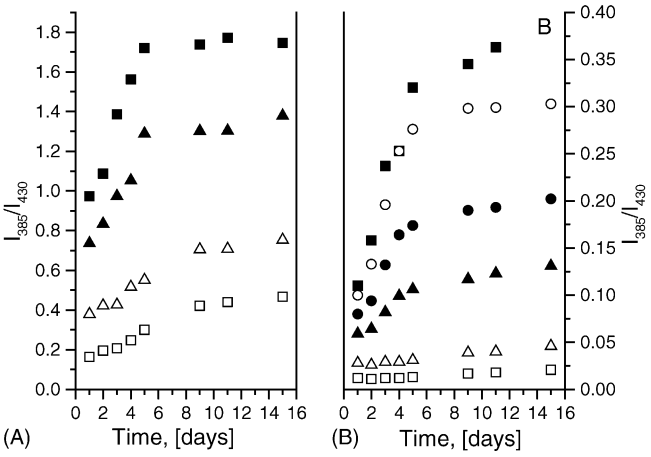

Fig. 9. Dependence of I_{385}/I_{430} for AP on the consecutive days of gelation for chosen excitation wavelength: (■) −313 nm, (○) −317 nm, (●) −321 nm, (▲) −325 nm, (△) −341 nm, (□) −357 nm; (A) $c_{AP} = 10^{-7}$ M, (B) $c_{AP} = 5 \times 10^{-6}$ M.

ing 4.5 with the use of aminopyrene at the concentration 10^{-7} M (Table 3). Both for λ_{ex} = 325 and λ_{ex} = 340 nm two-exponential kinetics of the probe fluorescence emission decay was reported. It is described by the relation

$$I(t) = A_1 \exp\left(\frac{-t}{\tau_1}\right) + A_2 \exp\left(\frac{-t}{\tau_2}\right) \quad (2)$$

where τ_1 and τ_2 are the lifetimes of aminopyrene recorded during silane sol gelation. The lifetime of a dominating component oscillated around 5 ns, typical of the inert form of aminopyrene [21]. At the excitation wavelength λ_{ex} = 340 nm, the amount of the probe with this lifetime was on the level of around 90% during the entire gelation process. The other lifetime was ascribed to the probe form that was a result of the interaction between a molecule and proton coming probably from the hydroxyl group. It increased from 31.5 ns in the starting sol to 57 ns in a highly viscous sol. An increase of the percentage of this domain and the lifetime shortened to 16 ns were observed in the gel. Similarly, at λ_{ex} = 325 nm on the consecutive days of the process two lifetimes of both domains were recorded with a clear tendency towards an increase of the content of the longer lifetime component in the stored gel. For comparison, it is known from literature that the lifetimes of aminopyrene adsorbed on the surface of silica MCB type gel (Matheson, Coleman, Bell), containing –OH geminal groups on the surface, are prolonged to 135 ns. The adsorption of AP on silica that has isolated, vicinal –OH groups (Fisher and Baker type) occurs without protonation and the lifetime of aminopyrene is 5 ns [13].

Table 3
Aminopyrene lifetime for sol–gel transition $c_{AP} = 10^{-7}$ M

λ_{ex} (nm)	State	Days of gelation	Lifetime			
			τ_1 (ns)	%	τ_2 (ns)	%
325	Sol	1	–	–	–	–
340			5.0	91.2	31.5	8.8
325	Sol	2	4.9	86.9	51.2	13.1
340			5.1	86.4	30.2	13.6
325	Sol	3	5.7	92.4	54.4	7.6
340			5.4	88.4	56.6	11.6
325	Sol–gel	4	5.8	86.1	42.2	13.9
340			5.5	85.2	45.8	14.8
325	Gel	5	6.0	72.9	17.9	27.1
340			4.8	90.8	32.8	9.2
325	Gel	8	4.8	64.5	15.6	35.5
340			–	–	–	–
325	Gel	10	5.01	57.5	16.3	33.8
340			–	–	–	–

The obtained results were compared with the results of time-resolution measurements for aminopyrene in water solutions at pH values varying between 1.8–12.0. Irrespective of pH of the environment, one inert form of aminopyrene of the lifetime 5 ns was found. This would confirm results of the steady-state studies, namely the protonated form of APH^+ obtained in a strongly acidic environment is unstable and is dissociated. On the other hand, the interaction between the probe molecule and proton during gelation and in the formed gel is stable.

4. Summary

Aminopyrene is a very good fluorescence probe for studying sol–gel transition in the silane gel. The application of AP concentration of about 10^{-7} M and excitation wavelength 313–330 nm allows us to observe by the fluorescence method an increased interaction between the molecule and proton coming most probably from the unbound hydroxyl groups of the monomer during the gelation process. It is reflected by the change in excitation and emission spectra probe and an increase of the lifetime of one of the domains, identified in the formed gel. This provides an evidence of the formation of a protonated stable form of APH^+ in the gel.

Additionally, the use of aminopyrene allowed us to estimate the concentration of hydroxyl groups unbound into the gel net and to determine the position of these groups against each other as of the geminal type.

Acknowledgements

The authors are grateful to the Committee for Scientific Research (Poland) for a financial support through grant no. 4 TO8E OO924.

References

[1] S. de Marcos, J. Galindo, J.F. Sierra, J. Galban, J.R. Castillo, Sens. Actuators 57 (1999) 227–232.
[2] R.-An Doong, H.-Ch. Tsai, Anal. Chim. Acta 434 (2001) 239–246.
[3] C. McDonagh, P. Bowe, K. Mongey, B.D. MacCraith, J. Non-Cryst. Solids 306 (2002) 138–148.
[4] V.G. Andreou, Y.D. Clonis, Anal. Chim. Acta 460 (2002) 151–161.

[5] A. Kumar, R. Malhotra, B.D. Malhotra, S.K. Grover, Anal. Chim. Acta 414 (2000) 43–50.
[6] S.A. Yamanaka, D.H. Charych, D.A. Loy, D.Y. Sasaki, Langmuir 13 (1997) 5049–5053.
[7] M.A. Doody, G.A. Baker, S. Pandey, F.V. Bright, Chem. Mater. 12 (2000) 1142–1147.
[8] E. Miller, J.S. Miller, Colloid Polym. Sci. 281 (2003) 745–753.
[9] E. Miller, J. Photochem. Photobiol. A 152 (2002) 249–257.
[10] C.D. Geddes, J.M. Chevers, D.J.S. Birch, J. Fluor. 9 (1999) 73–79.
[11] G.A. Baker, S. Pandey, E.P. Maziarz III, F.V. Bright, J. Sol–gel Sci. Technol. 15 (1999) 37–48.
[12] B.H. Milosavljevic, J.K. Thomas, J. Phys. Chem. 92 (1988) 2997–3001.
[13] P. Hite, R. Krasnansky, J.K. Thomas, J. Phys. Chem. 90 (1986) 5795–5799.
[14] C. Henneuse-Boxus, A. De Ro, P. Bertrand, J. Marchand-Brynaert, Polymer 41 (2000) 2339–2348.
[15] S. Pankasem, J.K. Thomas, J. Phys. Chem. 95 (1991) 7385–7393.
[16] E. Miller, Wiadomości Chemiczne 54 (2000) 437–455.
[17] N.J. Bonzagni, G.A. Baker, S. Pandey, E.D. Niemeyer, F.V. Bright, J. Sol–gel Sci. Technol. 17 (2000) 83–90.
[18] H. Schmidt, H. Scholze, in: J., Fricke, (Ed.), Proceedings of the First International Symposium on Aerogels (1st ISA), vol. 6, Wurzburg, Germany, 1985, p. 49.
[19] C.J. Brinker, G.W. Scherer, Sol–gel Science, Academic Press, 1990, p. 181.
[20] N.G.A. Baker, B.R. Wenner, A.N. Watkins, F.V. Bright, J. Sol–gel Sci. Technol. 17 (2000) 71–82.
[21] J.K. Thomas, E.H. Ellison, Colloid Interface Sci. 89 (2001) 195–238.