Fluorescence Monitoring of Polyacrylamide Hydrogel Using 4-Aminophthalimide

Anindya Datta, Swati Das, Debabrata Mandal, Samir Kumar Pal, and Kankan Bhattacharyya*

Department of Physical Chemistry, Indian Association for the Cultivation of Science, Jadavpur, Calcutta 700 032, India

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4-Aminophthalimide (4-AP) trapped in water-filled micropores of polyacrylamide (PAA) gel is studied using steady state and time-resolved emission spectroscopy. The position of the emission maxima and the temporal decay of 4-AP in the hydrogel indicate the presence of multiple microenvironments arising from the variation of the pore size. In PAA gel, the emission maximum of 4-AP exhibits a marked dependence on the wavelength of excitation and varies from 470 to 550 nm as λ_{ex} is varied from 300 to 400 nm. Global lifetime analysis at various wavelengths suggests the presence of two species of lifetime 1.34 and 7.21 ns with the relative amplitude of the short component increasing with an increase in the emission wavelength. The 550 nm emission band with a short lifetime (amplitude of the short component being 0.86) corresponds to an environment very similar to that of water. The 470 nm band with a much longer lifetime (amplitude of the long component, 0.85), however, implies a medium far less polar than water that does not allow the solvent-mediated proton transfer to occur. No dynamic Stokes shift is observed, which implies that even inside the semirigid gel the water molecules relax at a very fast rate (<60 ps). It is also observed that the rotational relaxation time of 4-AP in PAA gel is too fast to be detected in a picosecond setup.

1. Introduction

In recent years, water soluble polymers have generated considerable interest due to their versatile applications and biocompatibility.[1−13] The microenvironment of the aqueous polymer networks and the water molecules attached to them have been studied by various techniques such as light scattering,[3] fluorescence,[4] NMR,[5,9] dielectric relaxation,[6] calorimetry,[7−9] and also simulation.[13] The porous synthetic hydrogels have received particular attention due to their diverse applications as biomaterials, controlled-release devices, chromatographic packings, and electrophoresis gels.[1,7−12] The synthetic hydrogels, though inherently insoluble in water, can entrap a considerable amount of water within the polymer networks. The pore size in such semirigid systems can be varied by varying the concentration of the monomer (acrylamide).[10] Among the various types of hydrogels polyacrylamide (PAA) gel is most suitable for optical studies because it remains optically transparent for a wide range of concentrations of the monomer and the cross-linker. While in such a gel most of the molecules move freely and hence a fluorescent probe molecule experiences a solution-like environment, movement of a minute fraction of the probe molecules is markedly restricted.[1] Very recently, Moerner et al. studied the Brownian motion of such an immobilized *single* probe molecule, nile red, in polyacrylamide gel using far field microscopy.[1]

More recently, special attention has been given to the relaxation properties of water molecules present in unusual environments.[14,15] Ordinary water molecules exhibit solvation dynamics in the subpicosecond time scale.[14a] However, in certain organized media such as cyclodextrins,[14a] reverse micelles,[15a,b] or micelles[15a] water molecules relax in a time scale slower by several orders of magnitude. Since these organized media mimic biological systems, the relaxation behavior of such systems are of considerable interest. Inspired by these results, we have decided to probe the microenvironment of the

* E-mail: pckb@iacs.ernet.in. Fax: 91-33-473-2805.

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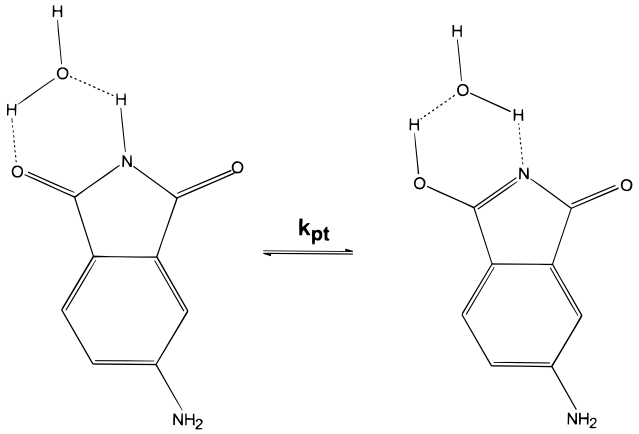
Scheme 1. Solvent-Mediated Proton Transfer in 4-AP



polyacrylamide gels using time-resolved fluorescence spectroscopy of a well-known solvent polarity probe 4-aminophthalimide (4-AP).[16−20] Recently, several authors have studied many aspects of polymer photophysics in aqueous medium using fluorescent probes.[4] In most of these studies, pyrene was used as the fluorescent probe and the information on the microenvironment was inferred from the ratio of the intensities of the different vibronic bands of pyrene emission, pyrene excimer emission, or energy transfer from pyrene to naphthalene. Unfortunately, fluorescent probes like pyrene provide no information on the dynamic properties of the water molecules present in the gel. 4-AP is particularly suitable for studying both the static and the dynamic properties of the gel for the following reasons. Firstly, the emission energy, quantum yield (ϕ_f), and lifetime (τ_f) of 4-AP are good indicators of the polarity or more precisely the hydrogen-bonding ability of the medium. The emission energy, quantum yield (ϕ_f), and lifetime (τ_f) of 4-AP decrease slightly with an increase in solvent polarity in aprotic solvents but quite markedly in protic solvents. For instance, λ_{em}^{max}, ϕ_f, and τ_f of 4-AP in dioxane are 435 nm, 0.73, and 15 ns, respectively, while the corresponding numbers in acetonitrile are 458 nm, 0.63, and 14 ns, respectively. However, in protic solvents the emission energy, ϕ_f, and τ_f decrease quite abruptly. For example, in water the λ_{em}^{max}, ϕ_f, and τ_f of 4-AP are 550 nm, 0.01, and 1.2 ns, respectively.[19,20] The marked reduction in ϕ_f and τ_f of 4-AP in protic solvents is attributed to an ultrafast solvent-mediated proton transfer process (Scheme 1)[18b,19] and the relatively small S_1-S_0 energy gap in the proton-transferred species (II) as a result of which its ϕ_f and τ_f decrease according to the energy gap law of nonradiative transitions.[19,21] Secondly, 4-AP has been widely used to study relaxation properties of different solvent molecules by an ultrafast dynamic Stokes shift.[16−19] The question that naturally arises is whether the gel exhibits solvation dynamics, i.e., how quickly the water molecules reorganize within the gel around a dipole, instantaneously created by exciting the probe 4-AP molecules by a picosecond pulse

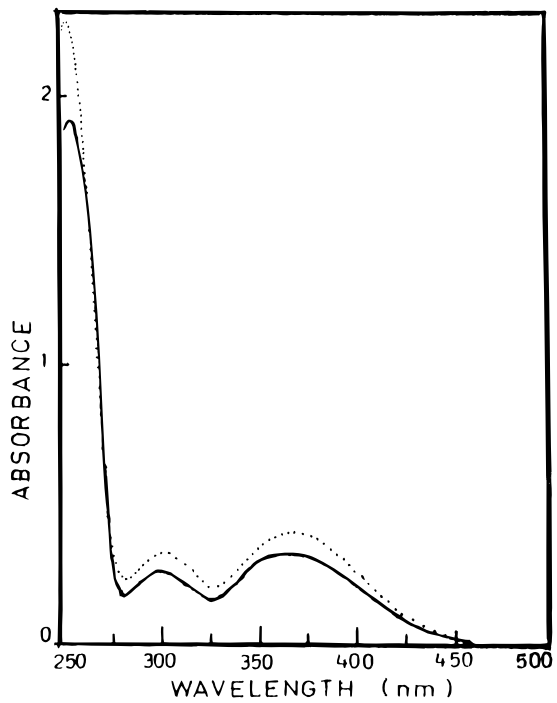


Figure 1. Absorption spectra of 8×10^{-5} M 4-aminophthalimide in (a) water (···) and (b) 5% polyacrylamide gel (—).

or whether the gel matrix allows the solvent-mediated proton transfer described in Scheme 1. In the present study we have addressed these issues by studying 4-AP in PAA gels using steady state and time-resolved emission techniques.

2. Experimental Section

4-AP (Kodak) was purified by repeated recrystallization from a 1:1 water−methanol mixture. The dilute (5%) polyacrylamide gel in aqueous medium was prepared by following the literature procedure.[22] To 2 mL of an 8×10^{-5} M aqueous solution of 4-AP taken in a quartz test tube were added 100 mg of acrylamide and 2.7 mg of *N,N′*-methylenebis(acrylamide). The solution was purged with nitrogen for 20 min. Then 20 μL of ammonium persulfate solution (containing 40 mg of salt in 1 mL of water) and 20 μL of sodium dithionite solution (containing 31 mg of salt in 1 mL of water) were added. The solution was quickly vortexed for thorough mixing and left undisturbed to polymerize for 24 h at 10 °C. For recording the absorption spectra, a reference gel was prepared in water without the probe, 4-AP. The laser system and the single photon counting apparatus are described in earlier publications.[15b,19] The response time of the setup is about 60 ps. The wavelength of excitation for the time-resolved studies is 300 nm.

3. Results

A. Steady State Emission Results. Figure 1 depicts the absorption spectrum of 4-AP in 5% PAA gel. It is readily seen that the absorption spectrum is remarkably similar to that of 4-AP in water[20] with the characteristic peaks at around 255, 302, and 362 nm except for the slight blue shift by 8 nm in gel compared to water. The similarity of the absorption spectrum of 4-AP in PAA with that in water suggests that 4-AP does not react with either the monomer or the cross-linker or the initiator. It should be noted that the blank gel does not absorb any light at wavelengths >300 nm and does not show any emission.

While the absorption spectrum of 4-AP in PAA gel is very similiar to that in water, the emission spectrum of 4-AP in gel exhibits striking differences from that in water. In water the emission maximum and ϕ_f of 4-AP are

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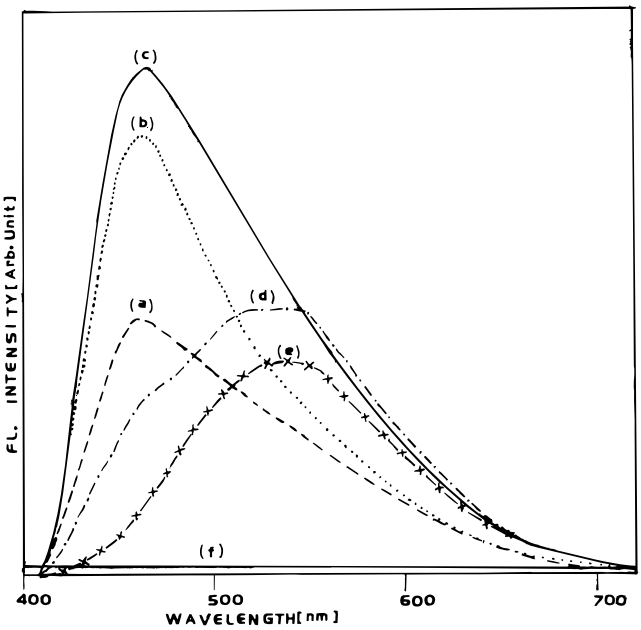

Figure 2. Emission spectra of 8×10^{-5} M 4-aminophthalimide in 5% polyacrylamide gel excited at (a) 300 nm (- - -), (b) 325 nm (···), (c) 350 nm (—), (d) 375 nm (— · —), and (e) 400 nm (×) and (f) emission spectrum of the reference gel excited at 300 nm (—).

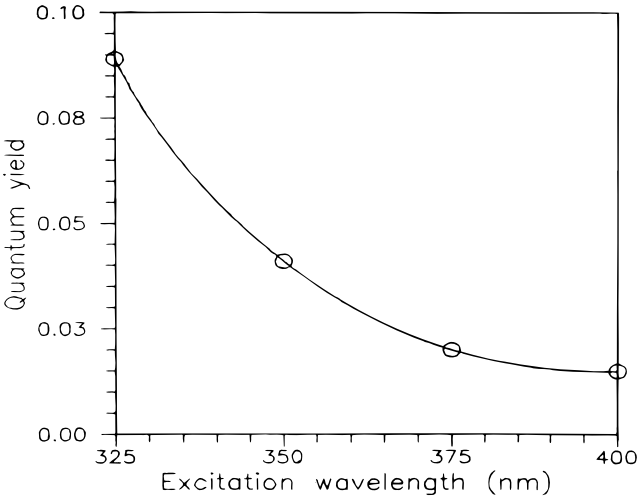

Figure 3. Variation in quantum yield of 8×10^{-5} M 4-aminophthalimide in 5% polyacrylamide gel with excitation wavelength.

Table 1. Excitation Wavelength Dependence of Emission of 8×10^{-5} M 4-AP in Polyacrylamide Gel

λ_{ex} (nm)	λ_{em}^{max} (nm)	ϕ_f
325	470	0.089
350	470	0.041
400	550	0.015

independent of the wavelength of excitation. In PAA gel, however, the λ_{em}^{max} and ϕ_f of 4-AP vary considerably with the wavelength of excitation (λ_{ex}). When 4-AP in the gel is excited at wavelengths shorter than 360 nm, the emission maximum of 4-AP is at 470 nm, while for excitation at the red end ($\lambda_{ex} > 385$ nm) the emission maximum is at 550 nm, which is very similar to that in water. At intermediate wavelengths (e.g., $\lambda_{ex} = 375$ nm) the emission spectrum is very broad (Figure 2). Figure 3 and Table 1 describe the variation of ϕ_f of 4-AP in gel with λ_{ex}. The ϕ_f of 4-AP in PAA gel decreases monotonically with an increase in λ_{ex} from 0.089 at $\lambda_{ex} = 325$ nm to 0.015 at $\lambda_{ex} = 400$ nm. Evidently, the marked excitation wavelength dependence of the emission spectra of 4-AP indicates a broad distribution of the microenvironments, i.e., microheterogeneity.

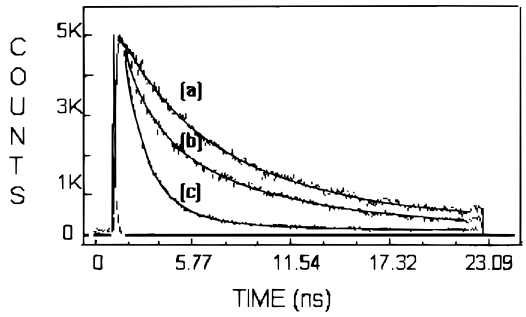

Figure 4. Fluorescence decays of 8×10^{-5} M 4-aminophthalimide in polyacrylamide gel at (a) 430 nm, (b) 500 nm, and (c) 600 nm.

Table 2. Decay Characteristics of Emission of 8×10^{-5} M 4-AP in Polyacrylamide Gel

λ_{em} (nm)	a_1	τ_1 (ns)	a_2	τ_2 (ns)	$\langle\tau\rangle^a$ (ns)	χ^2	% of fast emission[b]
430	0.20	1.34	0.80	7.21	6.04	1.17	4.44
470	0.15	1.34	0.85	7.21	6.33	1.15	3.18
500	0.49	1.34	0.51	7.21	4.33	1.31	15.16
520	0.78	1.34	0.22	7.21	2.63	1.17	39.74
550	0.86	1.34	0.14	7.21	2.16	1.00	53.35
580	0.91	1.34	0.09	7.21	1.87	1.14	65.21
600	0.94	1.34	0.06	7.21	1.69	1.13	74.53
630	0.94	1.34	0.06	7.21	1.69	1.47	74.53

[a] $\langle\tau\rangle = a_1\tau_1 + a_2\tau_2$. [b] $(a_1\tau_1/\sum_i a_i\tau_i)100$.

B. Time-Resolved Emission. In the PAA gel the fluorescence decays of 4-AP are found to be wavelength dependent. At the blue end (430−470 nm), τ_f of 4-AP is quite long, while at the red end (600−630 nm), it is much shorter (Figure 4 and Table 2). Such a wavelength dependence of the fluorescence decay is in sharp contrast to the time dependent Stokes shift observed in usual solvation dynamics experiments. For time dependent Stokes shift experiments, the fluorescence decay of 4-AP at the red end is usually observed to be relatively longer compared to that at the blue end and often exhibits a growth.[14−19] The absence of a time dependent Stokes shift for 4-AP in PAA gel suggests that even in the semirigid matrix the solvation of the probe by water molecules is too fast to be detected in our picosecond setup (response time 60 ps). We had also attempted to study rotational relaxation of the probe, 4-AP, in 5% PAA gel. However, the decays of the emissions at 470 and 550 nm for both parallel (0°) and perpendicular (90°) polarization are found to be identical, which indicates that in the PAA gel the rotational relaxation is faster than the system response time (60 ps).

Obviously, extracting meaningful rate constants in such a heterogeneous environment is quite difficult.[23] At the wavelength of excitation (300 nm), 4-AP molecules in different environments are excited simultaneously, as evidenced by the very broad emission spectrum (Figure 2). This results in a large distribution of lifetimes. Global lifetime analysis of all emission wavelengths reveals that all the decays can be fitted to biexponentials with two components of 1.34 and 7.21 ns, the relative amplitudes being different for the different wavelengths (Table 2). It is readily seen that with an increase in the emission wavelengths, the relative contribution of the fast component of emission increases (Table 2).

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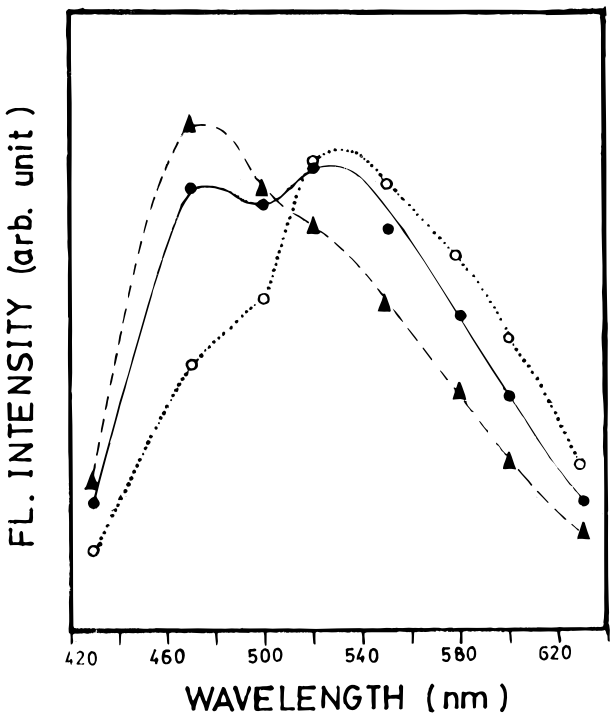

Figure 5. Time-resolved emission spectra of 4-AP at (a) 0 ps (○), (b) 1500 ps (●), and (c) 2500 ps (▲).

Using the best fits to the fluorescence decays, time-resolved emission spectra of 4-AP in polymer hydrogels have been constructed at different times (Figure 5). The results indicate that at short times the emission maximum is at ≈550 nm while at long times (>2000 ps) the emission maximum shifts to ≈470 nm. At intermediate times the time-resolved emission spectrum is a superposition of these two spectra.

4. Discussion

Both the steady state and the time-resolved studies indicate that 4-AP experiences multiple microenvironments inside the PAA hydrogel. This arises from the natural inhomogeneity of the gel matrix. As noted in earlier works, the PAA gel consists of a distribution of pore sizes and the mean pore size decreases with an increase in monomer concentration.[10] For 5% PAA gel the average pore diameter is 4 nm,[10] which is big enough to enclose the probe, 4-AP. Though assigment of each individual type of environment is nearly impossible, some quite interesting and useful generalizations can be made by concentrating only on the blue and and the red end of the emission spectrum and noting that the global lifetime analysis indicates the presence of two decay components of 1.34 and 7.21 ns.

Firstly, the very prominent emission band with a maximum at 550 nm obtained by exciting the gel at wavelengths greater than 385 nm is very similar to that of 4-AP in water while the lifetime (τ_f = 1.34 ns) of the major component of the decay (amplitude, 0.86) at 550 nm in the gel, is also close to τ_f of 4-AP in water (1.2 ns).[19] This suggests that in PAA gel many 4-AP molecules experience an environment similar to ordinary bulk water and undergo ultrafast solvent-mediated proton transfer (Scheme 1) to produce the proton-transferred species, II, from which the 550 nm emission originates.

Secondly, the very much blue-shifted emission with the λ_{em}^{max} at 470 nm obtained for wavelengths of excitation shorter than 360 nm is markedly different from that in water. The 470 nm band is blue shifted by 80 nm from the emission maximum of 4-AP in water and by 50 nm from that of 4-AP in other protic solvents (e.g., 520 nm in methanol[20] or formamide). The occurrence of an emission band at 470 nm in PAA gels indicates that the solvent-mediated proton transfer is completely prevented for some of the 4-AP molecules. This is a surprising result because the hydrogel environment contains a lot of water molecules and -$CONH_2$ groups, both of which can participate in the solvent-mediated proton transfer. The lifetime of the major component (amplitude, 0.80−0.85) of 4-AP in PAA gel at the blue end (430−470 nm), 7.21 ns, is about 6 times longer than that in water. The quantum yield of the 470 nm band is also much higher (0.089) compared to that in water (0.01). The emission maximum, lifetime, and quantum yield of the 470 nm band imply that in the hydrogel some of the 4-AP molecules experience an environment very much different from water. The emission maximum of 4-AP at 470 nm is actually close to those in acetone or acetonitrile. The observed τ_f (7.21 ns) which is 6 times longer than that of 4-AP in water is close to the reported τ_f of 4-AP in the α-cyclodextrin cavity.[20] It appears that in the interlaced network of the polymer fibers some of the 4-AP molecules get entrapped in such a small pore that the solvent-mediated ultrafast proton transfer is completely blocked. It may be recalled that Moerner et al. also reported that in PAA gel, while almost all the molecules of the probe, nile red, experience a water-like environment, a minute fraction (2%) of the probe molecules gets completely immoblized inside very small pores.[1]

The variation of the quantum yield of emission (ϕ_f) of 4-AP with the wavelength of excitation (λ_{ex}) in the PAA gel can be explained as follows. As noted earlier, the lifetimes (τ_f) of the major components of the decay of 4-AP in the gel at the blue and the red ends of the emission spectrum are in the ratio 7.21:1.34 = 5.4. Since the quantum yield $\phi_f = k_r\tau_f$, where k_r is the radiative rate constant, if we assume k_r is the same for all the environments, the quantum yield of 4-AP in the two environments should be of the ratio 5.4:1. On excitation at the red end (400 nm), mainly those 4-AP molecules in the water-like region are excited, giving rise to a ϕ_f of 0.015, which is close to that of 4-AP in water (0.01). At the blue end (325 nm) the 4-AP molecules in the less polar polymer network are excited preferentially to give a 6 times higher ϕ_f of 0.089. It is interesting to note that the measured quantum yield is of the ratio 0.089:0.015 or 6:1, which is close to that predicted from the lifetime values (5.4:1). The small difference arises from the fact that at each wavelength of excitation 4-AP molecules in different environments are excited simultaneously and it is difficult to ascertain what fraction of the light is absorbed by which kind of 4-AP molecule. As λ_{ex} increases, the contribution of the 4-AP molecules in the waterlike environment increases which gives rise to the gradual decrease in ϕ_f with increase in λ_{ex}.

The complete absence of the time-resolved Stokes shift indicates that the relaxation dynamics of the water molecules in the hydrogels occur on a time scale <60 ps and is thus too fast to be observed in our picosecond setup (response time ∼60 ps). Along with the global lifetime analysis, the time-resolved emission spectra (Figure 5) demonstrate the existence of two types of microenvironments, a water-like one responsible for the 550 nm emission maximum at short times and an aprotic one that gives rise to the long lived emission with a maximum at 470 nm.

The fact that the rotational relaxation of 4-AP in PAA gel is faster than 60 ps indicates that a vast majority of the 4-AP molecules reorient quite fast in the gel matrix.

It may be recalled that the time-resolved emission anisotropy study by Bright et al.[24a] of acrylodan-labeled proteins entrapped in orthosilicate sol−gel based biogel also indicates that the probe (acrylodan residue) and the protein itself remain remarkably mobile within the gel. Bright et al. reported that the subnanosecond local rotational motion of the probe remains essentially unchanged within the gel and on subsequent aging. More recently, using steady state emission anisotropy, Negri et al. showed that for titania gels at the sol−gel point while the bulk viscosity increases abruptly the fluorescence anisotropy does not change perceptibly.[24b] These observations along with the results of the present study that the rotational dynamics of 4-AP in the PAA gel is very fast demonstrate very high mobility of the probes in the gels.

5. Conclusions

The present work shows that 4-AP is a sensitive fluorescent probe for the microenvironment of the PAA gel. It is observed that there is a marked heterogeneity in the PAA gel arising mainly from the variation of the pore size. In the PAA gel, most of the 4-AP molecules experience a water-like environment. However, some of the 4-AP molecules get entrapped in very small pores with a polarity much less than that in water and in which the solvent-mediated proton transfer is completely prevented. No time-resolved Stokes shift is observed, which indicates that the relaxation time of water in the semirigid PAA gel is too fast to be detected in our TCSPC setup. Further, the rotational relaxation time of the probe, 4-AP is estimated to be less than 60 ps. The very fast solvation and the rotational dynamics suggest that the probe (4-AP) and the water molecules remain extremely mobile in the semirigid PAA gel.

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