



8-Anilino-1-naphthalenesulfonate (ANS) as a probe for Poly(vinyl alcohol)(PVA) swelling

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Abstract : Poly (vinyl alcohol) (PVA) is a hydrophilic polymer developed for biomedical applications. Swelling properties of PVA gels is very important for its drug delivery applications. There are many ways to study the swelling behaviour. Here we are proposing a fluorescent study by using 8-anilino-1-naphthalenesulfonate (ANS) as an extrinsic fluorescent probe. When ANS incorporated PVA films are allowed to swell in water, there is a loss of fluorescence intensity at 453 nm and there is a new peak at 500 nm. Thus, ANS is proposed as a fluorescent probe for the sensing of hydration of PVA.

Keywords : Fluorescence Spectroscopy, film, Poly (vinyl alcohol), ANS, Life time, Steady state fluorescence anisotropy.

1. INTRODUCTION:

A polymeric hydrogels is a 3 dimensional network of structure with full of water. Hydrogels are also generally highly biocompatible, which may be attributed to the high water content of hydrogels. Biodegradability or dissolution in case of hydrogels may be brought about by enzymatic, hydrolytic, or environmental (e.g. pH, temperature, or electric field) pathways; however, degradation is not always desirable depending on the time frame and location of the drug delivery device¹. The swelling behaviour of polymer hydrogels has been a topic of intense research because of various potential biomedical applications². Poly(vinyl alcohol) (PVA) is a hydrophilic polymer, has unique properties. PVA, shown in figure 1 is a particularly interesting polymer system because of its low toxicity, high biocompatibility and high degree of swelling in water³. PVA has high affinity towards water but chemically inert in many solvents. In humid atmosphere the tensile strength of PVA decreases. But water increases the plasticity of PVA and thus its tear strength and elongation⁴.

To study the polymer swelling, many techniques have been used and some of them are DSC, PNMR^{5,6}, AFM⁷ *etc.* Fluorescence molecular probes are increasingly found to be very useful in obtaining structural and dynamical information on a verity of organized and aggregate systems like micelles, lipid bilayer membranes, polymeric gels, cyclodextrin cavity, *etc.*

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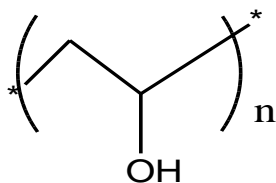


Figure 1: Structure of Poly (vinyl alcohol) PVA

ANS (8-anilino-1-naphthalenesulfonate) (ANS) shown in figure 2 is an extensively utilized fluorescent probe. It is weakly fluorescent in aqueous medium and preferentially distributes onto hydrophobic and hydrophilic interfaces, where they show a blue shift in emission⁸. Spectroscopic studies of ANS with PVA were interested because of ANS, give a blue shift of fluorescence emission maxima and the increase of fluorescence intensity and lifetime, having high hydrophobicity.

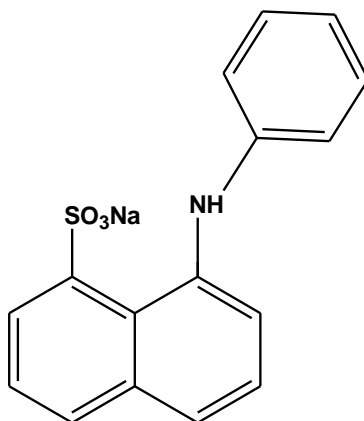


Figure 2: Structure of 8-anilino-1-naphthalenesulfonate (ANS)

Thus, in the present work we examine the swelling behaviour of PVA-ANS films by the fluorescence studies of the emission behaviour, fluorescent anisotropy changes and fluorescent lifetime of ANS. The results are explained in section 3.

2. Experimental section:

2.1 Materials:

Poly(vinyl alcohol) (PVA) (98-99)% hydrolysed with average molecular weight 31000-50000) purchased from AVRA. 8-anilino-1-naphthalenesulfonate (ANS) (> 95% hydrophobic fluorescent probe) purchased from Tokyo chemical industry, Japan. All the chemicals were used without further purification. Distilled water is used for sample preparation. Stock solution of 3.1×10^{-3} M ANS was prepared in methanol. The final concentration of ANS is 3×10^{-5} M in PVA films.

2.2 Sample preparation:

PVA films were prepared by continuous stirring of PVA in water 4 % (w/v) at 80 °C. After complete dissolution of PVA the solution is cooled to 40 °C and transferred to a Petri dish. These dishes were left 3-4 days in a dust free atmosphere to dry and form a self-supporting film. The PVA film was removed from the dish and used for further studies.

2.3 Method of Analysis:

The absorbance of the samples were measured by UV-Visible spectrophotometer JASCO (V-650) (range 200-900, resolution 1nm, band width 5nm).

The Steady-State Fluorescence measurements were carried out by using Jasco, FP-8500 spectrofluorimeter, with a Xenon arc lamp as the light source. Excitation and emission spectra were recorded with a slit width of 5nm and measurement range 300-600 nm. The scan speed was kept at 1000nm min⁻¹.

The fluorescence anisotropy (r_{ss}) values were obtained at the emission maxima using the expression

$$(r_{ss}) = (I_{\parallel} - GI_{\perp}) / (I_{\parallel} + 2GI_{\perp})$$

where I_{\parallel} and I_{\perp} refer to the fluorescence intensities when the emission polarizer is parallel and perpendicular, respectively, to the direction of polarization of the excitation beam and G is the factor that corrects for unequal transmission by the diffraction gratings of vertically and horizontally polarized light.

Fluorescence lifetime measurements were carried out by using a Fluoromax-4, spectrofluorimeter single-photon-counting fluorimeter in a time-correlated single photon-counting arrangement, consisting of a picosecond/ nanosecond LED (Horiba jobin yvon fluorocube fluorescence). The instrument response time was approximately 50 ps.

3. RESULTS AND DISCUSSION:

Figure 3 shows the UV-Visible spectra of PVA-ANS dry film with ANS concentration 3×10^{-5} M. The absorption peaks were observed at 284, 390 nm and used for the fluorescence studies.

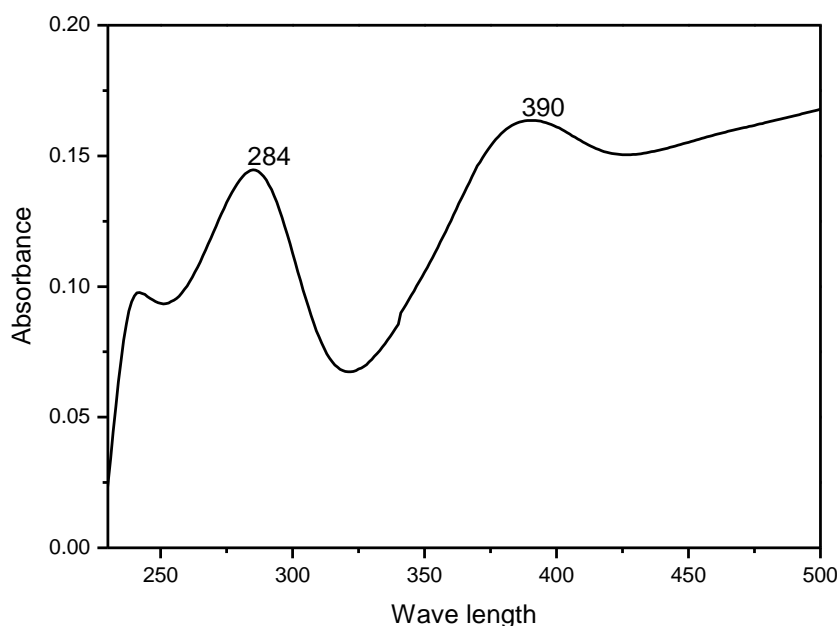


Figure 3: Absorption spectra of ANS in PVA film. [PVA]=4 % (w/v), [ANS] = 3×10^{-5} M

Fluorescence studies explain the behaviour of ANS in the PVA film and release due to swelling at room temperature. PVA has high affinity in water and it was identified that ANS is one of the most suitable probe to study the swelling of PVA-ANS film. This is because it has a very low fluorescence in water and it shows a significant increase in fluorescence intensity, lifetime, emission energy (blue shift), and steady-state fluorescence anisotropy on partitioning to the hydrophilic-hydrophobic interface. The increase of these fluorescence parameters is due to the combined effect of the decrease in the local polarity at the interface and restricted mobility of the probe molecule. The emission spectra of PVA-ANS film and ANS in water are shown in figure 4. The concentration of PVA is 4% (w/v) and ANS is 3×10^{-5} M. In water ANS gives a very weak fluorescence at 500nm in dry film the emission is very intense and observed at 453nm.

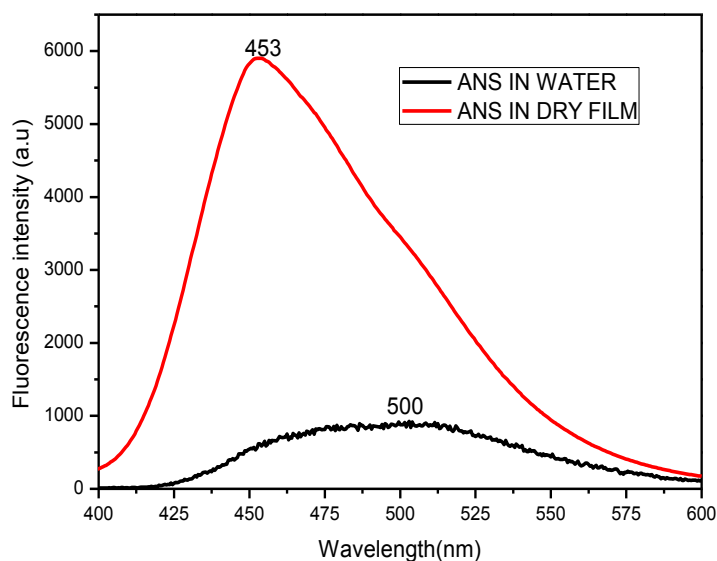


Figure 4: Fluorescence emission spectra of ANS in PVA dry film and water. [PVA] = 4%(w/v) and [ANS] = 3×10^{-5} M.

The variation of fluorescence intensity of ANS with time in PVA film on addition of water is shown in figure 5. After placing the film in water, fluorescence spectra were recorded every 5 minutes. It is seen that the intensity of the peak at 453 nm decreases and the appearance of a peak at 500 nm meter due to the release of ANS to the water. It is well known that at water ANS gives very weak fluorescence and the fluorescence intensity of the peak at 500nm is also decreases with time. The spectrum measured at 60 minutes clearly shown the absence of 453 peak indicates the absence of ANS in the film, which explains the swelling of PVA dry gel in water and release of ANS.

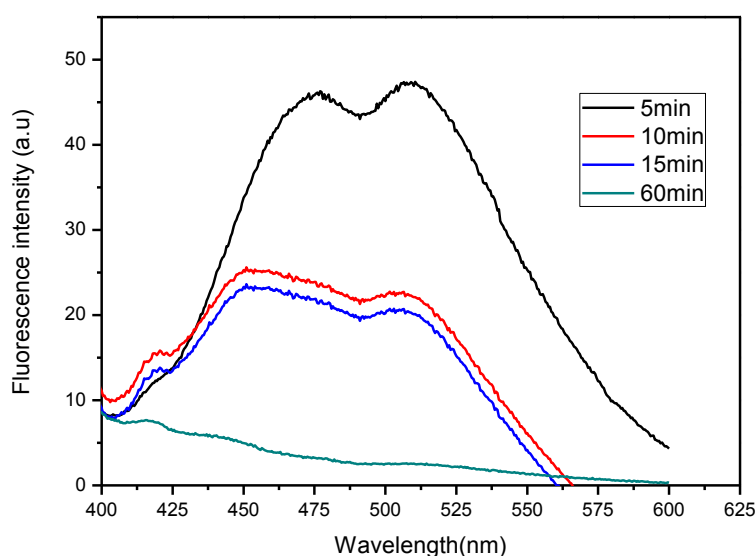


Fig 5 Fluorescence Emission peak 500nm of ANS in water, PVA 4%(w/v) and (ANS) 3.1×10^{-5} M.

Fluorescence anisotropy (r_{ss}) is a measure of the average angular displacement of the excited-state probe molecule in a microenvironment, which reveals the degree of rotational diffusion of the fluorescent probe

during its excited-state lifetime. The rotational diffusive motion of the molecule depends on the viscosity of the medium. Thus r_{ss} is expected to be a useful parameter in obtaining information on the efficiency of the rotational motion of ANS. Generally, the value of r_{ss} varies between the maximum of 0.1 for a completely restricted fluorophore (limiting r_{ss}) and the minimum of 0.0 for a completely free molecule⁹. Fluorescence anisotropy measurement has done for PVA-ANS dry film with an excitation wavelength at 274 nm and emission wavelength at 450 nm. The value obtained for measurement is 0.09. The reported steady-state fluorescence anisotropy of ANS in water is 0.03. The value observed for dry film clearly shows that the ANS is in a well-organized and rigid atmosphere.

Table 1: The fluorescence lifetime values of PVA-ANS samples at room temperature.

| Time(ns) | α_1 | $\tau_1(\alpha_1)$ | α_2 | τ_2 | α_3 | τ_3 | Average(τ) | χ^2 |
|--------------------|------------|--------------------|------------|----------|------------|----------|-------------------|----------|
| dry film | 0.04 | 11.8 | 0.35 | 3.87 | 0.55 | 1.26 | 2.68 | 0.99874 |
| 5 min swollen film | 0.24 | 6.17 | 0.51 | 1.80 | 0.28 | 0.53 | 2.47 | 0.99829 |

Table1 shows the fluorescence lifetime values of PVA dry film and 5 minutes swollen in water samples at room temperature. The fluorescence decay profiles, obtained by excitation at 370 nm, shows a good fit for three exponential decay components for both the samples. An average lifetime was calculated by taking the weighted average lifetime (τ_{av}) of the three-component decay given by

$$\tau_{av} = \frac{\tau_1(\alpha_1) + \tau_2(\alpha_2) + \tau_3(\alpha_3)}{\alpha_1 + \alpha_2 + \alpha_3}$$

where α_i are the amplitudes of each component in percent. The calculated τ_{av} of ANS decreases with swelling. The value decreased from 2.68 to 2.47 in a five minutes water contact. It is reported that the average life time of ANS in water is 0.25ns by Pal *et al*. The decrease of the average fluorescence lifetime roughly corresponds to the decrease in the fluorescence intensity of 453nm peak. This clearly explains the swelling of PVA films and the shift of ANS from the well organised atmosphere to more free water environment.

PVA is polymer shows saturation swelling behavior, have enough non-polar interchain interaction. It does not dissolve completely even keep a long time in water. This swelling behavior will be useful for many applications like loading and release of drugs, molecules *etc*.

ANS is a suitable probe to study this swelling and release because of its two different emission properties in organized media and water. In ANS incorporated PVA films, ANS is expected to remain associated with the polymer matrix. ANS is leaching to water during swelling because of its anionic nature. For a verification of this explanation, and see the morphology of dry and swollen films the SEM images were taken. The SEM images of dry and swollen PVA films are shown in figure 5 (a) and (b) respectively.

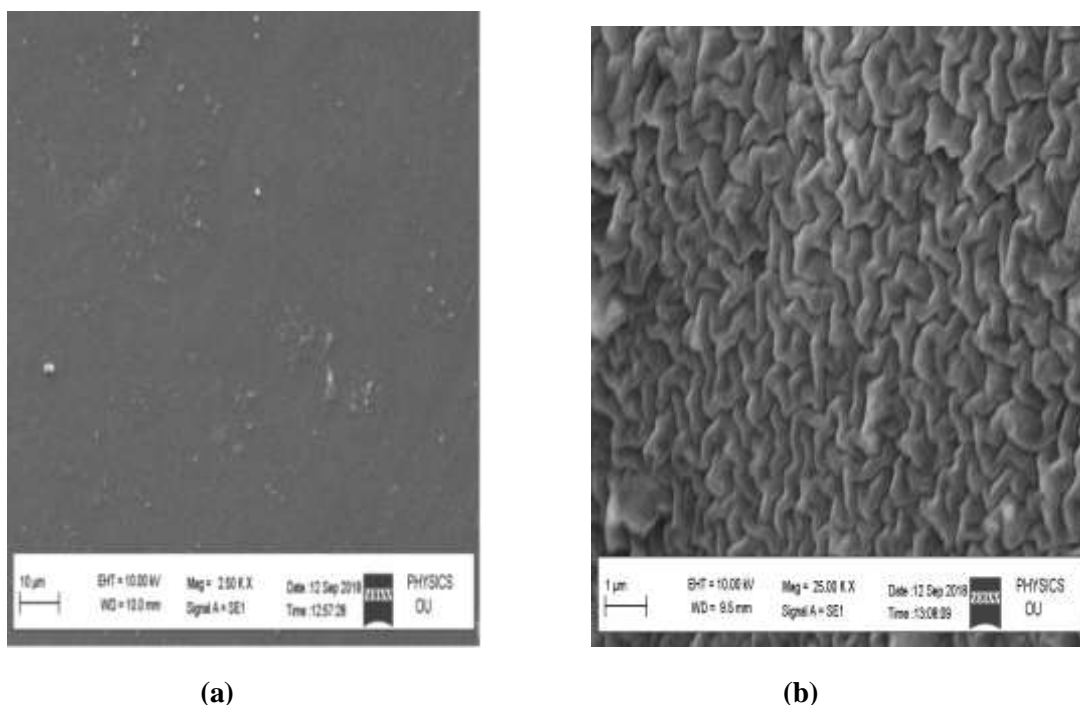


Figure 5: SEM images of PVA (a)dry and (b)completely swollen film

From the SEM images it is very clear that PVA is very uniformly swelling in presence of water and the incorporated molecules can release easily to the water medium.

Thus, all three fluorescence parameters, fluorescence spectral shift, intensity changes, fluorescence anisotropy and lifetime data, consistently report the release of ANS from the PVA matrix to water during swelling. The SEM images support the Fluorescence study.

4. CONCLUSION:

The swelling behavior is very important to delivery applications. Fluorescence is a simple technique to study the swelling properties of polymeric gels. When ANS incorporated PVA films were allowed to swell, the fluorescence intensity of 453 nm peak decreases and started showing the appearance of 500nm peak due to release to water.

The change in different fluorescence parameters (intensity, emission wave-length, anisotropy) explains the swelling and release of ANS from the PVA matrix to water. The SEM strongly supports the Fluorescence studies.

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