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Quantitative, Non-Contact Measurements of the Physicochemical Properties of Thermoresponsive Polymers using Fluorescence Methods.

by

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Abstract

In biomedical science and engineering the use of microscale thin polymer films is widespread. These polymer films are often formed on complex geometries which can provide problems for in-situ analysis and the accurate measurement of multiple physiochemical properties of thin polymer films is critical in understanding the polymer function and performance. When undertaking physicochemical characterisation of thermoresponsive polymers we must consider polymer structure and also the environment created by a polymer when it is used as a thin film. In addition, we need to account for the infiltration of solvents and other factors which may mediate the polymer behaviour. Water uptake in a polymer film can lead to significant changes in the physiochemical properties. This in turn can affect mechanical properties and may lead to such problems, in medical devices, as reduced adhesion, pronounced physical and chemical aging and swelling and expansion, compromising the intended function of the polymer and hindering biocompatibility. Thus there is a need for a non-contact, non-destructive method of analysis of these polymer films. Fluorescence spectroscopy offers a potential non-contact method for the in-situ analysis of thin polymer films. In this work we demonstrate the use of the dual-band emission, 3-hydroxyflavone based fluorophores for monitoring the hydration process in thin (~ 10 µm) films of poly(N-isopropylacrylamide) (PNIPAm), and poly(NIPAM-co-ethylpyrrolidone-methacrylate) (poly(NIPAM-co-EPM) copolymers. The fluorescence of 3-HF probes is very sensitive to changes in local polarity and hydrogen bonding. This is observed as shifts and changes in the relative intensity of the two emission bands (N* and T*), which originate from an excited state intramolecular proton transfer (ESIPT) process. The spectroscopic parameters of these probes can thus be correlated with the physiochemical properties of the probe microenvironment. We incorporated the probes into films of the thermoresponsive polymers and studied their fluorescence emission behaviour at different levels of humidity. Analysis of the spectral data showed that with increasing humidity, the ratio of two emission bands (log(I_N*/I_T*)) for all
fluorophores increases. Changes were also observed in position of two emission bands; as humidity increases the N* band shifts to the red with respect to the T* band. It was also observed that the spectral intensity varied with increasing humidity. When measurements were made above and below the lower critical solution temperature (LCST), it was clear that the films were still absorbing water above the LCST. Fluorescence measurements also revealed that these probes are also very sensitive to the subtle differences in copolymer composition as the fraction of EPM is increased. Results indicate a more equilibrated hydration process with the incorporation of EPM. Polarity measurements performed (using the solvatochromic Reichardt’s betaine dye) revealed that increasing water content in the polymer films resulted in an increase in polarity (which would be expected due to the highly polar nature of water). However, little or no variation in band positions or E_T(30) parameter indicated that polarity did not vary with changes in chemical composition, with the exception of PEPm films where a red shift of the absorption band was noted indicating a decrease in polarity. However a non-linear response of band position with increasing RH for PEPm indicated that solvent sorting was taking place.
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1 Introduction

1.1 Polymer Systems.

A polymer can be defined as “A large molecule comprising of covalently linked repeating monomer units of similar or identical chemical structure” [1] Polymers are long-chain molecules with very large molecular weights measured in the hundreds of thousands and therefore the term “macromolecules” is very often used to refer to these materials. [2] Natural polymers such as starch, wool, and cotton were the first polymers in use, however in the early twentieth century the production of synthetic polymers began. [2] The first synthetic polymers of importance (Bakelite and nylon) showed the impressive possibilities of these new materials, and it was thought at the time that synthetic polymers could be good substituents for natural polymers; however synthetic polymers have long since become important materials in their own right. [2, 3] Polymeric materials are often chosen for a variety of applications and uses including in: building and construction, transport, packaging, toys, sport, clothing, furniture, electrical and medical etc. They can also be used as plastics, rubbers, fibres, coatings, adhesives, foams or speciality polymers.[3] It would be incorrect to say “name the application and polymers can do it”, however their uses are continuing to expand into an increasing number of fields. [3] Unlike low-molecular-weight compounds, polymers possess unique cooperative properties; and it is these unique properties that form the basis of their success. [1]

1.2 Hydrophilic Polymer Systems.

Hydrophilic polymer systems are a group of polymers that are characterised by their compatibility with and solubility in water, their properties being a direct result of their chemical composition. [4] The presence of oxygen in the polymer chain or as a side group contributes to hydrophilicity due to its high electronegativity and two pairs of free electrons, contributing to hydrogen bonding (H-bonding) with neighbouring molecules. When water molecules come into contact with hydrophilic polymers they penetrate between adjacent polymer chains and separate them and this is due to the
similar polarities of water and pendant molecules on a polymer chain, and also due to H-bonding. [4] Examples of hydrophilic polymer systems include acrylics (acrylamides and acrylates), amine functional polymers, vinyl acids and vinyl alcohols. Within biomedical science and engineering, the use of microscale thin polymer films is widespread, with roles in tissue engineering, drug delivery systems, microfluidic devices, bioadhesion mediators and bioactuators. [5-18] To name one of many potential uses of thin polymer films it would be as coatings on drug eluting coronary stents, where not only does the polymer act as a drug reservoir (providing antirestenosis therapy) but it also acts as a biocompatibility modulator to improve device performance. [8, 10, 19, 20] Such polymer coatings are relatively thin (from µm down to nm) and are formed on complex geometries which may provide problems for in-situ analysis. Another important consideration is the fact that these coatings have large surface area to mass ratios and water uptake is an important factor to consider. Therefore, the choice of polymer for such applications is very important, as coating stability, device efficacy and long-term storage are all dependant on the physiochemical properties of the polymer. [21] One area of significant interest has been the development of synthetic functional polymers that display stimuli-responsive behaviour with the intention of providing “smart” applications (Figure.1.) in the biomedical field. [5, 22, 23]
1.3 Stimuli-Responsive Polymer Systems.

From the dawn of polymer science as a discipline one area of significant interest has been the design and development of synthetic functional polymers that respond to external stimuli. [5, 22-24] This class of synthetic polymers have been designed often to mimic biopolymers, and a variety of functional forms have been developed to meet various specific industrial and scientific applications. [25] Over the years these polymers have acquired many different names, for example: “environment sensitive polymers” [26], “stimuli-responsive polymers” [27], “intelligent polymers” [28, 29], or “smart polymers” [22, 30]. “Smart” polymers can be defined as materials that undergo strong conformational changes in response to small but significant changes in
the surrounding environment. [22, 25] In other words these polymers are materials that undergo sharp, large, reversible non-linear conformational changes due to the application of a small external stimulus; and such stimuli responsive behaviour can be attributed to the balance of hydrophilic and hydrophobic groups in the polymer system. [1, 5, 22, 23, 27, 31-34] The consequent responses may be observed as changes in surface characteristics, shape, solubility etc. [25] “Smart” polymers can be classified into three classes depending on their physical forms (Figure.2.) : (i) linear free chains in solution (following the application of an external stimulus the polymer undergoes a reversible collapse), (ii) covalently crosslinked gels and reversible or physical gels (for which swelling or shrinking behaviour is environmentally triggered), and (iii) chain adsorbed or surface-grafted form (following a modification of the external parameter the polymer reversibly swells or collapses on a surface). [25] The response of these “smart” polymers can be induced by a variety of environmental triggers, for example ionic strength, light, magnetic field, pH, electric field and temperature. [5, 15, 22-24, 31-33, 35-41] From a biomedical standpoint, the favoured “smart” polymers are generally those sensitive to pH and/or temperature changes. [31] The most widely studied class of stimuli responsive polymers are thermoresponsive polymer systems; as the name suggests, these systems undergo conformational changes in response to temperature.[23, 31]
1.3.1 Thermoresponsive Polymers

Thermoresponsive systems display a critical solution temperature, at which a phase change of the polymer system occurs in accordance with the polymer composition. Polymers that display an increase in their water solubility with increasing temperature are described as displaying an upper critical solution temperature (UCST) or a higher critical solution temperature (HCST); in other words, such polymer systems appear monophasic above and biphasic below a certain temperature. On the other hand, polymer systems that display the opposite possess what is known as a lower critical solution temperature (LCST) (i.e. the polymer becomes less solvated on increasing temperature exhibiting one phase below a certain temperature and phase separation above it). Below the LCST, the polymer is soluble in aqueous solution due to the domination of hydrophilic interactions (i.e. hydrogen bonding between the polymer and water), and it assumes a relaxed coil-like conformation. Raising the temperature above the LCST results in the collapse of the polymer system due to the domination of hydrophobic interactions, minimising the polymer’s contact with water molecules and eventually leading to its precipitation from solution, as the polymer now exists in a more globule-like conformation (Figure 4).
Figure 3: Schematic representing the coil-to-globule transition of a thermoresponsive polymer system in aqueous solution as the temperature is elevated from below the LCST to above. [5, 45]

Here we shall consider the 2\textsuperscript{nd} law of thermodynamics to explain this phenomenon:[42]

\[
\Delta G_m = \Delta H_m - T\Delta S_m
\]

where \(\Delta G_m\) is the Gibb’s free energy of mixing, \(\Delta H_m\) is the enthalpy of mixing and \(\Delta S_m\) is the entropy of mixing. For a process to be spontaneous, the Gibb’s free energy term should be negative. In the case of a polymer solution which possesses a UCST the entropy of mixing is usually large and positive but is dominated by the enthalpic contribution at low temperatures. When the temperature increases, the entropic contribution increases, and eventually surpasses the enthalpic contribution at the UCST, ultimately resulting in negative Gibb’s free energy. Therefore in these polymer systems, higher temperatures enhance solubility.[42]

Conversely, for polymer solutions possessing a LCST, H-bonding between polymer polar groups and water molecules are the driving force for solvation at low temperatures, resulting in a large, dominant, negative enthalpy of mixing. In this state the polymer is ordered leading to an unfavourable negative entropy factor, but overall the system is stable in this mixed form below its LCST due to the favourable enthalpic contribution. Heskins and Guillet have suggested that the phase separation at the LCST of a polymer can be attributed to entropy.[46] At higher temperatures the contribution from entropy surpasses the exothermic enthalpy contribution from
hydrogen bonding between polar groups in the polymer and water molecules.[5, 31, 33, 47] It is this balance between entropy and enthalpy that causes the polymer to assume a more hydrophobic state above the LCST.

Four of the most common groups of thermoresponsive polymers are: (i) poly(N-alkyl substituted acrylamides) e.g. poly(N-isopropylacrylamide) (PNIPAm) which displays a sharp LCST at 32°C [25, 46] and (ii) poly(N-vinylacrylamides) e.g. poly(N-vinylcaprolactam) (PVCL) which has an LCST of about 38°C.[25, 44] PNIPAm and PVCL exhibit LCST behaviour in a physiologically relevant range rendering them viable for biomedical applications. Other thermoresponsive polymer systems include: poly(N,N-diethylacrylamide) (PDEAAm) with a LCST ranging from 25-32°C, poly(N,N-dimethylaminoethyl methacrylate) (DMAEMA) with a LCST of about 50°C, and poly(N-n-propylacrylamide) (PNPAm) which has a LCST of 25°C.[5, 26, 44, 48] It is also possible to modulate and fine tune the LCST by the addition of additives to, or by co-polymerisation of these core thermoresponsive polymers. For example, the addition of sodium dodecylsulfate increases the LCST, whereas addition of sodium chloride has the opposite effect.[49, 50] Alternatively copolymerisation with hydrophilic or hydrophobic co-monomers can be used to increase or decrease the LCST respectively.[37]
Table 1: LCSTs of popular thermoresponsive polymer systems. Data taken from ref. [44]

A list of popular thermoresponsive polymer systems with their corresponding LCSTs is given in Table 1. Often these core polymers are used to synthesise co-polymers with specific thermoresponsive and other desired properties. For example poly(ethylene oxide) (PEO) and poly(propylene oxide) (PPO) have been used to fabricate block copolymers which possess an inverse thermoresponsive behaviour. These block copolymers are available commercially under the names Pluronics® and Tetronics®, many of which have been approved by the FDA and EPA for use in food, pharmaceuticals and agriculture.[5, 25, 26] (Figure 4).
1.3.1.1 Poly(N-isopropylacrylamide) (PNIPAm) and its copolymers

Poly(N-isopropylacrylamide) (PNIPAm) is the most widely studied thermoresponsive polymer. PNIPAm is a chemical isomer of poly-leucine, in that it has the polar peptide group in its side chain rather than in the backbone.[51] PNIPAm consists of a hydrocarbon backbone with hydrophilic and hydrophobic moieties in the form of carboxyl, amide and isopropyl groups respectively, Figure 5. In aqueous solution PNIPAm displays a LCST of 32°C; at this temperature it undergoes a sharp and reversible coil-to-globule phase transition from a hydrophilic to a more hydrophobic state, forcing water from the matrix.[6, 9, 14, 23, 33, 52-54] This phenomenon occurs due to the domination of entropic effects (displacement of water from the polymer matrix) over enthalpic effects (formation of hydrogen bonds between polymer and water molecules) as the temperature increases above the LCST.[31, 47] Below the LCST, PNIPAm chains exist in an extended coil conformation, and solvation is driven by the enthalpic gain from intermolecular hydrogen bonding between the PNIPAm chains and water molecules.[23, 55]
Solvation is further driven by a type of hydrophobic hydration, where the water molecules surround the non-polar isopropyl entities in a cage-like structure.[56] As the temperature is increased towards the LCST, intramolecular hydrogen bonding between carboxyl and amide groups on the PNIPAm chains result in the interruption of hydrogen bonding of these groups with water molecules, ultimately resulting in the chain adopting a collapsed conformation, driving out the water, and causing the polymer to precipitate out of solution.[55] These interesting properties of PNIPAm and its copolymers make them applicable to a diverse range of pharmaceutical and biomedical applications.[10, 13]

![Chemical structure of PNIPAm](image.png)

**Figure 5: Chemical structure of PNIPAm.[26]**

The thermal properties of PNIPAm can be modified by the introduction of hydrophobic (e.g. N-tert-butylacrylamide) (N-tBAAm) or hydrophilic (e.g.acrylamide) (AAm) groups to its molecular chains.[30] By copolymerisation with more hydrophobic monomers, like N-tBAAm, the LCST of the polymer is shifted to lower temperatures, while copolymerisation with more hydrophilic monomers, like AAm, increases the LCST (Figure 6).[30]
Rochev and co-workers have synthesised a series of co-polymers based on N-isopropylacrylamide (NIPAM) and N-tert-butylacrylamide (N-tBAAm) (Figure 7). Increasing the amount of the hydrophobic monomer (from a mole ratio of 0 to 50%) increases the co-polymer hydrophobicity and therefore lowers the LCST (from 33 to ~10°C) [57] (Figure 8). It was also observed that cell adhesion, cell growth properties and drug elution from cast NIPAM/N-tBAAm copolymer films were all dependant on the co-polymer composition.[58, 59]
Figure 8: LCST of poly(NIPAM-co-N-tBAAm) copolymers as a function of N-tBAAm contents. Taken from Ref [57]

In contrast, by increasing the amount of a hydrophilic monomer AAm in the NIPAm/AAm copolymers (from a molar ratio of 100:0 to 75:25), the LCST is increased (from 33 to 47°C) due to the increased hydrophilic nature of these copolymer systems with increasing AAm content (Table 2).[60]

Table 2: Changes in the LCST as a function of AAm content. Taken from ref [60]

1.4 Thermoresponsive Polymer Characterisation

When we consider the physicochemical characterisation of thermoresponsive polymers we have to consider, first the polymer itself and second the environment created by a polymer when it is deployed as a particle or a thin film. In the first case we are mainly looking at the chemical structure and conformation of the polymer,
often in solution, whereas in the second case we have to consider very carefully the infiltration of solvents and other factors which can mediate the chemical behaviour of the polymer. As previously mentioned, in many applications polymer coatings are very thin and are formed on complex geometries which can provide problems for *in-situ* analysis. Thus there is a need for the non-contact, non-destructive analysis of these types of thermoresponsive polymer films. Spectroscopy and in particular fluorescence based methods with the combination of high sensitivity and low probe concentration offer the best solution for these analytical challenges.

### 1.4.1 Physiochemical Characterisation by Solvatochromism

Measuring polarity and hydrogen bonding changes in thermoresponsive polymers is vital for understanding how these polymers will behave in the real world. The most widely based method for assessing polarity is based on solvatochromism, and there is an extensive literature on solvent systems. Solvatochromism describes the change in shape, location and sometimes intensity of absorption or emission spectra, relative to the polarity of the medium.[61-63] A hypsochromic (or blue) shift corresponds to negative solvatochromism, while a bathochromic (or red) shift corresponds to positive solvatochromism with increasing solvent polarity.[62] Solvatochromic methods are generally robust and reasonably easy to implement but do require the use of relatively high probe concentrations[64] (typical weight ratios of 36-20 to 1).[65] The most common solvatochromic solvent polarity scales are: the $E_T(30)$ scale of Dimroth and Reichardt[62], the $\alpha$ and $\beta$ scales of Kamlet and Taft[66, 67], the $\pi^*$ scale of Kamlet, Abboud and Taft[68] and the pyrene (Py) scale of Dong and Winnik[69]. These solvatochromic methods are typically used to characterise solvent systems but they have been extended to study polymer systems.

The $E_T(30)$ scale uses the 2, 6-diphenyl- 4- (2,4,6- triphenyl- 1-pyridino)- phenolate betaine dye (also known as Reichardt’s dye, figure 9).[62, 70] The $E_T(30)$ polarity parameter is based on the transition energy for the longest wavelength absorption band measured in the relevant environment and expressed in kcal·mol⁻¹.
\[ E_T(30) \ (\text{kcalmol}^{-1}) = \hbar c \nu_{\max} N_a = 2.859 \times 10^3 \nu_{\max} = \frac{2859}{\lambda_{\max}} \]

where \( \nu_{\max} \) is the wavenumber (cm\(^{-1}\)) and \( \lambda_{\max} \) is the wavelength corresponding to the transition, \( \hbar \) is Planck’s constant, \( c \) is the velocity of light and \( N_a \) is Avagadro’s number.[62] A normalised scale \( (E_T^N) \) was defined from 0 to 1 using tetramethylsilane and water as the extreme nonpolar and polar solvents respectively.[62, 70]

![Solvatochromic probe](image)

**Figure 9:** Solvatochromic probe (2,6-diphenyl-4-(2,4,6-triphenyl-1-pyridino)-phenolate betaine dye) used to define the \( E_T(30) \) scale of solvent polarity.[62]

The \( \alpha \) and \( \beta \) scales developed by Kamlet and Taft provide a measurement of solvent hydrogen-bond donor (HBD) or a hydrogen-bond acceptor (HBA) ability. These scales are based on a solvatochromic comparison method (SCM) which involves the comparison of solvent induced shifts of the longest wavelength absorption band of two similar compounds (\( i \) and \( j \)). For determination of \( \alpha \) values one compound cannot act as a HBA towards HBD solvents (e.g. 4-nitroanisole) whereas the other can (e.g. Reichardt’s dye).[66, 67, 70] To determine \( \beta \) values one compound cannot act as a HBD towards solvents (e.g. 4-nitro-N,N-dimethylaniline) whereas the other can (e.g. 4-nitroaniline).[70]

The solvent dipolarity/polarizability \( \pi^* \) scale provides a quantitative measure of the non-specific part of van der Waals interactions between solvents and solutes.[68, 71, 72] The original scale was based on the spectral properties of carefully selected
aromatic molecules which contain both electron-acceptor and electron-donor groups.[71, 72] Dimethyl sulfoxide (DMSO) and cyclohexane (c-C$_6$H$_{12}$) were used as reference solvents by taking $\pi^*(c$-C$_6$H$_{12}) = 0$ and $\pi^*$ (DMSO) = 1.[71, 72] More recently, the scale has been updated/revised and is now based on the averaging of data from several solvatochromic indicators.[73] Laurence et al. re-determined $\pi^*$ values for 229 solvents using only two solvatochromic indicators, 4-nitroanisole and N,N-dimethylamino-4-nitroaniline.[62, 73] For the physicochemical characterisation of polymers by optical spectroscopy, one can employ either vibrational or electronic spectroscopies. One of the key advantages of electronic spectroscopy either absorption or fluorescence is that they can be very sensitive, enabling the observation of subtle effects in condensed media. One of the simplest approaches is to follow a solvatochromic approach and measure the UV-visible spectra of the appropriate indicators.[65, 74, 75] Matsuguchi et al. employed solvatochromic methods to characterize the water sorption behaviour in polymer films.[65] They found that indicator band position for dry films was dependant on the kind of polymer, and its molecular weight (decreasing the molecular weight of PEO resulted in an increase of the $E_T(30)$ parameter). From the solvatochromic parameters obtained it was deduced that poly(ethylene oxide) (PEO) is an amphiprotic polymer. On increasing relative water vapour pressures, solvatochromic parameters were seen to increase; $\beta$ values were lower at higher water vapour pressures for PEO. The authors also examined the relationship between the $E_T(30)$ scale and the $\pi^*$, $\alpha$ and $\beta$ parameters and a linear correlation was observed indicating that the observed parameter reflects the microenvironment in both wet and dry films.

Szczupak et al. evaluated the micro-polarity and H-bond donor/acceptor ability for a series of thermoresponsive N-isopropylacrylamide/N-tert-butylacrylamide (NIPAm/NtBA) copolymer films using the $E_T(30)$, $\alpha$, $\beta$ and $\pi^*$ empirical solvatochromic parameters.[21] For the dry NIPAm/NtBA copolymer films it was found that they are strong H-bond acceptors ($\beta$), moderate H-bond donors ($\alpha$) and are strongly dipolar/polarizable ($\pi^*$). It was observed that $E_T(30)$, $\alpha$ and $\pi^*$ values all decreased linearly on increasing the hydrophobic NtBA fraction in the copolymer
films whereas in contrast the $\beta$ parameter was found to be relatively unchanged on increasing the fraction of NtBA (figure 10). Finally the authors also found a good correlation between experimental $E_T(30)$ and independently determined $\alpha$, $\beta$ and $\pi^*$ values which confirms that the behaviour of the solvatochromic indicators in the NIPAm/NtBA films is similar to that in solvents.[21]

Figure 10: Dependence of $E_T(30)$ polarity parameter, dipolarity/polarizability parameter ($\pi^*$), H-bond donor ability ($\alpha$ parameter) and H-bond acceptor ability ($\beta$ parameter) of poly(NIPAM-co-NtBA) copolymer films on increasing %NtBA component. Taken from ref [21]
1.4.2 Physiochemical Characterisation by Fluorescence

While the application of solvatochromic methods for the characterisation of physicochemical properties is feasible, and can generate accurate data, there is still a need for a measurement methodology that is more flexible. Specifically, for many applications, the concentrations required of the solvatochromic probes for absorption spectroscopy in these condensed media is relatively high (typical weight ratios of 36-20 to 1),[65] and it would be preferable to utilise much lower concentrations such as the levels employed in fluorescence spectroscopy (typical weight ratios of 1900-2230 to 1).[76]

Fluorescence is the phenomenon by which a molecule absorbs a photon of light and re-emits it as a longer wavelength, lower energy photon. The processes that lead from the absorption of light to fluorescence emission are best described by means of a Jablonski diagram (Figure11).
Figure 11: Jablonski diagram and illustration of the relative positions of absorption, fluorescence and phosphorescence spectra. VR, IC and ISC stand for vibrational relaxation, internal conversion and inter-system crossing respectively. $h\nu_A$, $h\nu_F$ and $h\nu_P$ represent energy absorbed, emitted as fluorescence and emitted as phosphorescence respectively. Reproduced from ref.[63, 77]

The majority of molecules exist in the lowest vibrational energy level of the ground state $S_0$ at room temperature. Following the absorption of a photon of light a molecule is elevated to one of the vibrational energy levels of either $S_1$ or $S_2$, the level which becomes populated is determined by the magnitude of the absorbed energy.
This process is very fast and occurs within $10^{15}$ seconds, which, according to the Frank-Condon principle, is too short a time for any displacement of nuclei to occur.[78] In the next $10^{14}$-$10^{-11}$ seconds, the excited molecule rapidly relaxes to the lowest vibrational energy level (the 0 level) of the $S_1$ singlet state; a process known as vibrational relaxation (and internal conversion if the singlet excited state is $S_2$).[63, 78] The molecule remains in the 0 energy level of the $S_1$ singlet state for a few tens of picoseconds to a few hundreds of nanoseconds (depending on the type of molecule and the surrounding medium) before emitting a photon of light (fluorescence) or undergoing inter-system crossing to the $T_1$ triplet state.[63] The energy of the emitted photon is lower, and thus of longer wavelength, than the absorbed photon due to energy loss in the excited state caused by vibrational relaxation. This difference in energy between the absorbed and emitted photon is called the Stokes shift and is an important parameter which can provide information on the environment of excited states.[63]

Other than internal conversion and fluorescence, there is a third possible de-excitation process known as inter-system crossing. This is a non-radiative transition in which molecules undergo a spin conversion from the $S_1$ to the $T_1$ state. Emission of photons from the $T_1$ state is called phosphorescence; phosphorescence is longer lived (milliseconds to seconds) and of lower energy than fluorescence and hence takes place at longer wavelengths (Figure 11). The transition from the $T_1$ triplet state to the $S_0$ singlet ground state is forbidden, however in certain instances spin-orbit coupling may be large enough to permit it.[63, 78]

Fluorescence measurements may be classified into two categories: steady-state and time-resolved measurements. The most common type of measurement is the steady-state measurement in which continuous illumination and observation are employed. In other words, the sample is illuminated with a constant beam of light and the emission spectrum is recorded.[78] Fluorescence occurs on a nanosecond timescale, thus when a sample is exposed to light steady state is reached almost instantaneously.[78]

Measured at a fixed excitation wavelength an emission spectrum is the
wavelength distribution of the fluorescence intensity, and may be presented on either a wavenumber (cm$^{-1}$) or wavelength (nm) scale. Fluorescence intensity ($I_F$) of a given sample is quantitatively dependant on the intensity of the incident light ($I_O$), fluorescence quantum yield ($\Phi_F$) and on the sample absorbance such that:

$$I_F = \Phi_F I_O (1 - e^{-\varepsilon \ell c})$$

where $\varepsilon$ is the molar extinction coefficient, $l$ is the optical path length and $c$ is the concentration.[79]

The second type of fluorescence measurement is the time-resolved measurement where the sample is excited by a pulse of light, and the pulse is generally shorter than the decay time of the sample. This type of measurement is used to measure intensity or anisotropy decays.[78] It should be noted that there is a relationship between steady-state and time-resolved measurements in that the steady-state observation is basically an average of the time-resolved over the intensity decay of the sample; for example: for a fluorophore with a single decay time ($\tau$) and a single rotational correlation time ($\Theta$), the intensity decay can be given by the following:[78]

$$I(t) = I_0 e^{-t/\tau}$$

where $I_0$ is the intensity at $t=0$ immediately following the excitation pulse. The steady-state intensity ($I_{SS}$) is given by:[78]

$$I_{SS} = \int_0^{\infty} I_0 e^{-t/\tau} dt = I_0 \tau$$

The value of the $I_0$ parameter depends on fluorophore concentration, therefore, in molecular terms, the steady-state intensity is proportional to the lifetime.[78] Fluorescence lifetime ($\tau$) can be defined as the average amount of time a molecule spends in the excited state before returning to the ground state, thus $\tau$ can be
expressed as the inverse of the total depopulation rate:[78, 80]

\[ \tau = \frac{1}{k_r + k_{nr}} \]

Where \( k_r \) is the rate constant for radiative deactivation with emission of fluorescence and \( k_{nr} \) is the overall non-radiative rate of depopulation defined as the sum of rate constant for internal conversion and intersystem crossing.[63] The lifetime becomes the inverse of \( k_r \) in the absence of non-radiative relaxation \((k_{nr}=0)\), and so is often referred to as the natural lifetime \((\tau_N)\). The natural lifetime \( \tau \) and the recorded quantum yield \( \Phi_F \) can be calculated from:[78]

\[ \tau_N = \frac{\tau}{\Phi_F} \]

There are two primary methods for measuring time-resolved fluorescence or lifetime-the time-domain and the frequency domain methods. In the time-domain method the sample is excited with a pulse of light and in the frequency domain the sample is excited with intensity modulated light. In principle, both methods can yield equivalent data for a wide range of experimental samples and conditions.[80]

Due to the fact that it determines the time available for the excited fluorescent molecule to interact with its microenvironment, fluorescence lifetime is one of the most important parameters of a fluorophore.[78]

**1.4.2.1 Environmental Factors Affecting Fluorescence**

Fluorescence emission is very sensitive to the microenvironment surrounding the fluorophore. This is especially true in condensed systems like polymers where multiple factors may be operating at the same time (Figure 12). This may result in changes in absorption / fluorescence intensity, shifts in absorption / fluorescence spectra, anisotropy, or fluorescence lifetimes.[81-83] Due to this high sensitivity of a fluorophore to its specific microenvironment, the fluorescence measurement method
has become very attractive in monitoring processes in polymer systems and polymer thin films. Fluorescence is a powerful tool for monitoring microscopic changes in such systems due to the high sensitivity and selectivity, short response time and non-destructive nature of the measurement.[81]

![Figure 12: Some environmental factors which may affect fluorescence.](image)

1.4.3 Fluorophores for Thermoresponsive Polymer Analysis.

1.4.3.1 Pyrene

Pyrene (Py) is a common fluorophore used for polarity assessment because the intensities of the various forbidden vibronic bands are very sensitive to solvent polarity.[63, 84] Karpovich and Blanchard explained that the relative changes in vibronic band intensities of the pyrene fluorescence spectrum are a result of vibronic coupling between the first (weakly allowed) and the second (strongly allowed) excited singlet states.[63, 85] Their observations also suggest that solvent-dipole-solute induced dipole interactions play a major role in giving rise to the pyrene scale.[85]
The extent to which an induced dipole moment is formed by vibrational distortions of the nuclear coordinates of pyrene is governed by the polarity of the solvent.[63, 85] The polarity of the pyrene environment may be estimated by measuring the ratio of the fluorescence intensities of the third and first vibronic bands (I$_I$/I$_{III}$) (Figure 13).[63, 84] Dong and Winnik developed the Py scale of solvent polarities, based on this ratio and the scale is relatively insensitive to the hydrogen bonding ability of protic solvents.[62, 63] Py values (I$_I$/I$_{III}$) range from 0.58 for n-hexane to 1.95 for dimethyl sulfoxide (DMSO).[62, 63] Changes in the pyrene lifetime can also be used to monitor changes in thermoresponsive polymers.[86]

![Image](image-url)

Figure 13: (a) Chemical structure of Pyrene, (b) Fluorescence spectra of pyrene in solvents of different polarity, taken from ref [84]

Szczupak et al. assessed the polarity of poly(NIPAm-co-NtBA) copolymer films by means of fluorescence based methods, using pyrene as a polarity sensitive fluorescent probe.[76] The authors report a decrease in the I$_I$/I$_3$ ratio of pyrene with increasing NtBA fraction indicating a reduction in polarity with increasing NtBA content (Figure 14), which is in agreement with previous results obtained in a solvatochromic study involving the same polymers.[21, 76]
However the authors concluded that the pyrene ratio displayed poor correlation with measured solvatochromic polarity parameters, [21] and this was attributed to the fact that the polarity component sensed by pyrene is only related to dipole-induced dipole interactions. This ignores the very significant H-bonding effects which are present in these films. Furthermore, the physiochemical changes that occur at the LCST of these co-polymers did not greatly affect pyrene fluorescence. This was shown by a linear decrease in the $I_1/I_3$ ratio for the thin films between 20 and 40 °C. For the PNIPAm used here, the decrease was from 1.345 to 1.285 over this temperature range which contrasts with the situation reported for aqueous solutions of PNIPAm where the ratio changes decreases from 1.79 to 1.38.[87] We also note that this magnitude of change is almost identical to that observed for solutions of pyrene in ethanol and 1-propanol.[88] This occurs because there is no distinct aqueous phase, so most of the Py probe remains dispersed throughout the solid polymer and, therefore there is much less change in emission properties. It should be noted that these measurements were made without rigorous humidity control [76], and these films probably contained a significant amount of adsorbed water. Based on these.
observations, pyrene was not recommended as a suitable polarity probe for thin film studies.

Winnik utilised pyrene labelled PNIPAm to study the temperature induced phase transitions in aqueous solutions.[76, 87, 89] One study showed that the photophysics of the pyrene when covalently labelled with PNIPAm is complex, is dependant on the degree of labelling, and involves emission from monomers and excimers.[87] At ambient temperature in water, the presence of ground-state pyrene dimers and higher aggregates is observed for the pyrene labelled PNIPAm. These aggregates can form between pyrene attached to the same chain or between pyrene probes located on different chains. Heating solutions of the labelled PNIPAm above the LCST results in a disruption of the pyrene aggregates and the quantum yield of the monomeric species increases relative to that of the excimers. This dissociation is complete in the case of the sparsely labelled polymer(PNIPAm/Py/200) but only partial in solutions of the more highly labelled polymer (PNIPAm/Py/20). When trace amounts of the labelled PNIPAM were added to solutions of unlabelled PNIPAm, changes in pyrene fluorescence could be used to ascertain the interaction between chains. They found that below the LCST, that there was no indication of interactions between labelled and unlabeled polymers, but, above the LCST, the labelled polymers are incorporated into the PNIPAm-rich phase. These fluorescence studies also showed that in the low concentration limit (<1 ppm) and for highly labelled polymers there was evidence for the formation of single-polymer micelles. Chee et al. have also investigated in detail the interactions between PNIPAm and pyrene using time resolved fluorescence spectroscopy and concluded that above the LCST PNIPAm is capable of solubilising hydrophobic guests such as pyrene but that below the LCST much of this capability is lost.[90]

1.4.3.2 ANS

ANS (1-anilino-8-naphthalene sulfonate) (Figure 15) was first discovered by Weber and Lawrence in 1954 and is one of the most well known polarity probes that
is highly fluorescent in low polarity solvents but is weakly fluorescent in aqueous solution.[63, 91] This important feature enables one to visualize only the hydrophobic region of a given system with minimal influence from ANS molecules remaining in the aqueous environment, and thus has found widespread application in biological and material sciences.[92-97]

![Chemical Structure of 1-anilino-8-naphthalene sulfonate (ANS)](image)

Figure 15: Chemical Structure of 1-anilino-8-naphthalene sulfonate (ANS)[63]

Kujawa et al. have utilised ANS to study the concentration (0.02 to 10 g/L range) and temperature dependant solution properties of telechelic PNIPAm (C_{18}-PNIPAM-C_{18}).[63, 98] As polymer concentration was increased a steep increase in emission intensity of ANS was observed, accompanied by a blue shift of emission band maxima. These observations with increasing polymer concentration indicate the increased hydrophobicity of the probe (ANS) environment (Figure 16).[98]

![Figure 16: (a) Emission spectra of ANS in water and in aqueous solution of telechelic PNIPAm at 20°C (b)Fluorescence intensity and position of emission band maxima as a function of polymer concentration at 20°C. Taken from ref [98]](image)
These fluorescence results support the results from DLS that suggest for the telechelic PNIPAm in solution, the degree of aggregation within the rosettes increases as the concentration increases, and that the steric crowding results in expulsion/release of polymer bound water in order to accommodate the increased steric pressure. When the emission is measured over the 10° to 50°C temperature range one observes at approximately 29°C a sharp increase in fluorescence intensity coupled with a blue shift in the emission band maximum (Figure 17). This is clear evidence for a very significant change in the micropolarity sensed by ANS, as with increasing temperature ANS passes from a hydrophilic environment of “highly hydrated rosettes”, formed at lower temperatures to the “hydrophobic medium of collapsed and associated polymeric micelles”. [98]

Figure 17: Temperature dependence of the fluorescence intensity and position of emission band maxima in polymer solution (polymer concentration is 0.1gL⁻¹). Taken from ref [98]
1.4.3.3 Two-band ratiometric fluorophores based on 3-hydroxyflavone

A common disadvantage of single band fluorescent probes is that the response can be affected by other factors (unrelated to the microenvironment) such as photobleaching, excitation source instabilities and variations in probe concentration; all of which may lead to unreliable measurements, particularly in viscous/rigid polymers.[81, 99-101] Fluorescent probes that exhibit dual band fluorescence emission, in which the ratio of the two emission bands is sensitive to environmental factors, have a clear advantage over single band fluorophores.[102, 103] Dual band fluorescence data does not depend on instrumental factors (i.e. illumination stability) or on probe concentration.[104-107] Additionally, these probes may be considered multiparametric due to the possibility of recording a number of spectroscopic variables which display sensitivity to different kinds of interactions with the microenvironment.[108-111] Molecules which undergo excited-state intramolecular proton transfer (ESIPT) display dual band fluorescence. ESIPT involves an intramolecular proton transfer of a hydroxyl proton to a carbonyl oxygen (or of an amino proton to a pyridinic nitrogen) via a hydrogen bond. The product phototautomer (T*) of the ESIPT reaction displays differences in structure and electronic configuration in comparison to its corresponding normal (N*) form; thus T* displays a fluorescence spectrum dramatically shifted to lower energies (Figure 18).[112, 113]
A variety of compounds that exhibit ESIPT have been reported however, it is only 3-hydroxychromones and their derivatives 3-hydroxyflavones, which display solvent dependant dual emission, that is not coupled to the presence of two or more ground- or excited-state conformers, or to a slow ESIPT rate.[104, 107, 114-120]

One of the most extensively studied ESIPT systems is 3-hydroxyflavone (3HF).[121-152] The first to identify the dual fluorescence of 3HF were Sengupta and Kasha and they described it as being due to emission from the normal (N) form and the tautomer (T) form.[143] The sequence of events that results in the two emission bands is the following (Figure 18): Absorption of a photon of light results in the normal Frank-Condon excited state which then relaxes to the N* state. The N* state can then undergo ESIPT to the T* state followed by emission of a photon of light resulting in the population of the ground T state with proton back transfer to the ground N state, which closes the cycle.[109] Both steady-state and time-resolved
spectroscopy have been extensively used to study ESIPT in 3-hydroxyflavone in a variety of solvents. The polarity, protic nature and temperature of the surrounding medium have been found to strongly affect the dynamics of ESIPT.[132, 151]

The main disadvantages associated with the use of 3-hydroxyflavone as an effective fluorophore are: (i) its absorption occurs in the ultraviolet ($\lambda_{\text{max}} = 343\text{nm}$ in EtOH), (ii) it has a low fluorescence quantum yield ($\Phi = 0.024$ in EtOH) and (iii) its N* band has a low relative intensity compared to the T* band in most media.[153] However, significant improvement of these parameters may be achieved through chemical modification of the fluorophore.[154-156] Modification of the chemical structure of the probe may adjust it to a specific range of solvent polarities and may switch of sensitivity to hydrogen bonding.[110, 153, 157-159]

Attachment of an electron donor dialkylamino group at the 4’ position of 3HF (Figure 19) is one such modification, which results in a large excited state dipole moment in the normal form which leads to a charge transfer character for normal fluorescence.[112, 160-162] The N* band is well resolved and its relative intensity is much higher, even in non-polar aprotic solvents, and it has also been shown that the N* band position and its intensity are dependant on solvent polarity. In contrast, the tautomer T* emission is less sensitive to solvent changes, due to the proton transfer, causing a smaller dipole moment of the T* form and a different orientation to that of the N* form.[112, 160, 162-166]
Figure 19: Examples of 4’-dialkylamino-substituted 3-hydroxyflavone derivatives. (a) 4’-dimethylamino-3-hydroxyflavone (b) 4’-diethylamino-3-hydroxyflavone (FE) (c) 5,6-benzo-4’-diethylamino-3-hydroxyflavone (BFE) (d) 4’- diethylamino-3-hydroxy-7-methoxyflavone (MFE).[112, 153, 158]

Based on the Onsager theory of liquid dielectrics (derived from dielectric polarization) the N* emission band maximum position should change linearly with solvent polarity $f(\varepsilon)$. [109, 162] This has been demonstrated for 4’-diethylamino-3-hydroxyflavone (FE) (Figure 20); a linear correlation between solvent polarity function $f(\varepsilon)$ and the N* band position can be seen (small deviations to the red can be attributed to the H-bond donor ability of the selected solvents). [109]
Figure 20: Plot of the positions of the emission band maxima in FE vs solvent polarity function $f(\varepsilon)$: ○ neutral solvents, □ H-bond acceptor solvents, ▲ protic solvents, △ and ▽ correspond to chloroform and dichloromethane respectively which are neutral solvents with considerable H-bond donor ability. Taken from ref [109]

In contrast to what has been reported for the parent 3HF, the ESIPT reaction in 4'-dialkylamino substituted 3-hydroxyflavones has been found to be reversible and significantly slower (ps for 4’dialkylamino derivatives as compared to fs for the parent 3HF).[167-169] Even so, when compared to radiative and non-radiative excited-state deactivations, the process is much faster and the observation of two emission bands (N* and T*) is due to the rapid formation of a dynamic equilibrium between the two states. Changes in the relative intensities are brought about due to interactions with the microenvironment which causes strong disruptions in the equilibrium.[169, 170] Due to significant differences between the N* and T* states, the intensities of the emission from the two states ($I_{N^*}$ and $I_{T^*}$) can be considered as independent variables. The ratio of intensities of these two states ($I_{N^*}/I_{T^*}$) is an important parameter which is connected to the relative energies of the N* and T* states and has been found to be a sensitive indicator of polarity.[109] Use of
4′-dialkylamino-3-hydroxyflavones allows the determination of a set of spectroscopic parameters which can differently characterize the physical properties of the microenvironment.

Klymchenko and Demchenko performed a detailed spectroscopic study of 4′-diethylamino-3-hydroxyflavone (FE) (Figure 19(b)) in 21 representative solvents.[109] The most important solvent-dependant changes were observed in the intensity ratio \( I_{N*/I_{T*}} \) of the two emission bands. A plot of the log ratio \( \log(I_{N*/I_{T*}}) \) of the two emission bands vs. the sum of the band positions \( \nu_{N*} + \nu_{T*} \) demonstrates separate parallel linear segments for protic and aprotic solvents on the \( \log(I_{N*/I_{T*}}) \) scale (Figure 21).[109] The conclusion as to whether an unknown environment or system is protic or aprotic may be made by the closeness of the point obtained for the unknown system to the corresponding linear segment; if the point lies between the parallel linear functions then this should be considered as a case of partial H-bonding.[109]

![Figure 21: log(I_{N*/I_{T*}}) vs. \( \nu_{N*} + \nu_{T*} \) for protic (▲) and all other (○) solvents. Taken from ref [109]]
The spectroscopic behaviour of the N* and T* emission bands depends strongly on the specific structure of the fluorophore, and structural modifications can modulate sensitivity to H-bonding and solvent polarity.[110, 153, 158] For example, in the case of 5,6-benzo-4'-diethylamino-3-hydroxyflavone (BFE) (Figure 19 (c)), the intramolecular hydrogen bonding with protic microenvironments can be switched off or eliminated due to the attachment of an additional benzene ring at the 5,6 position which sterically hinders H-bonding of the protic environment at the 4-carbonyl position, but there is still a possibility of a H-bond interaction between the microenvironment and the 3-hydroxy group.[158] BFE demonstrates a good linear correlation of \( \log(I_{N*}/I_{T*}) \) with the solvent polarity function \( f(\varepsilon) \), which is independent of the nature of the solvent (whether it be protic or aprotic) (Figure 22).[158]

**Figure 22:** Plot of \( \log(I_{N*}/I_{T*}) \) vs solvent polarity function \( f(\varepsilon) \) for 5,6-benzo-4'-diethylamino-3-hydroxyflavone (BFE). Adapted from ref [158]

It is only when there is an equilibrium between the N* and T* forms that the \( I_{N*}/I_{T*} \) and \( \log(I_{N*}/I_{T*}) \) parameters can operate as quantitative polarity measurements.[109, 169] Determining if the equilibrium has been reached can be
done by analysing a plot of $\log(I_{N^*/I_{T^*}})$ vs. band separation ($\nu_{N^*-\nu_{T^*}}$) (Figure 23). If a data point deviates from the linear regression it implies that ESIPT equilibrium cannot be reached and is indicative of some specific interaction between the probe and the microenvironment.[109, 169]

Figure 23: $\log(I_{N^*/I_{T^*}})$ vs. band separation ($\nu_{N^*-\nu_{T^*}}$) for 4-diethylamino-3-hydroxyflavone (FE). ○ neutral solvents, □ H-bond acceptor solvents, ▲ protic solvents, △ and ▽ correspond to chloroform and dichloromethane respectively. Taken from ref [109]

Due to the unique properties of 3HF and its derivatives, they have become useful in a variety of applications including sensing water content of acetone and in reverse micelles, as probes of protein-binding sites, as potential laser dyes, as hydrogen bonding sensors and in the analysis of thermoresponsive polymer thin films.[76, 164, 171-176]

Szczupak et al. assessed the polarity of poly(NIPAm-co-NtBA) copolymer films by means of fluorescence based methods, using 3-hydroxyflavone derivatives (FE, MFE and BFE) as polarity sensitive fluorescent probes.[76] The hydrogen-bonding
effects inherent in the PNIPAm thin films also produces problems for the use of 3-hydroxyflavone derivatives for polarity assessment of thermoresponsive hydrophilic polymers in thin films, using simple emission parameters. This arises from the heterogeneity of the ground-state H-bonding and an ESIPT process that is not in equilibrium, both effects can be elucidated from the observed excitation wavelength dependence and a difference in the fluorescence lifetimes of the N* and T* bands.[76, 109, 169] There are two possible H-bonding interactions between the polymers and FE/MFE; the first being between the 4-carbonyl group of the fluorophores and the amide hydrogen of the polymer and the second between the 3-hydroxy group of the fluorophores and the carbonyl group of the polymer. In the case of BFE the strongest H-bond interaction is between the 3-hydroxy group of the fluorophore and the carbonyl group of the polymer as the possibility of a H-bond interaction at the 4-carbonyl group of the polymer is sterically hindered due to the additional benzene ring in the 5,6-position. The authors suggest that this H-bonding interaction in the ground state may lead to disruption of the intramolecular H-bonding in the 3-hydroxyflavone fluorophores and formation of the new emissive species that do not undergo ESIPT.[76]

1.5 Objectives of the Study.

Within biomedical science and engineering the use of microscale thin polymer films is widespread with applications in tissue engineering, drug delivery systems, microfluidic devices etc. Water uptake in a thin polymer film can lead to significant changes in the physicochemical properties of a polymer. These changes will in turn affect mechanical properties,[100, 177] and this may lead, in medical devices, to such problems as reduced adhesion and mechanical properties, pronounced physical and chemical aging and swelling and expansion, compromising the intended function of the polymer and also hindering biocompatibility. In hydrophilic polymers like PNIPAm, there is always likely to be an appreciable amount of water infiltrating the polymer if it is handled under ambient conditions and precautions are not taken to
exclude water infiltration. The situation will be exacerbated when using thin films as the surface area is much larger which facilitates water uptake.

The objective of this body of work was to develop a non-contact, non-destructive measurement to characterise the effect of water sorption on the physicochemical properties of thin, thermoresponsive polymer films of poly(N-isopropylacrylamide) and poly(NIPAm-co-ethylpyrrolidone methacrylate) using fluorescence methods.
2 Materials and Methods

2.1 Materials

Poly(N-isopropylacrylamide) (PNIPAm) with an average molecular weight of 20,000-25,000 was purchased from Sigma-Aldrich and used as received. The PNIPAm was stored without taking any particular precautions (e.g. it was stored under ambient conditions without controlling humidity). Co-polymers of NIPAM and ethylpyrrolidone-methacrylate (EPM) with weight ratios (w/w) of 100:0 ($M_w = 10,000$), 90:10 ($M_w = 12,000$), 80:20 ($M_w = 13,500$), 70:30 ($M_w = 16,000$), 60:40 ($M_w = 15,000$) and 0:100 ($M_w = 32,000$), (NIPAM:EPM) were provided by Dr. Carlos Elvira, Institute of Polymer Science and Technology, CSIC, Madrid, Spain. The NIPAM-EPM copolymer chemical structure is shown in figure 24. The PNIPAm received from Dr. Elvira has a molecular weight of 10,000 (compared to 20,000-25,000 for commercial PNIPAm). For the purposes of comparison the commercial PNIPAm will be referred to as PNIPAm1 and the synthesised PNIPAm will be referred to as PNIPAm2.

![Chemical structure of poly(N-isopropylacrylamide-co-ethylpyrrolidone-methacrylate) (NIPAM-EPM) copolymer.](image)

The LCST’s of the co-polymers were determined by UV-vis turbidimetry and
micro-DSC measurements; for copolymers NIPAM:EPM 100:0 – 60:40 the LCST’s were determined using UV-vis turbidimetry measurements and the LCST of pEPM was determined using micro-DSC (table 3 and figure 25). These results were provided by Dr. Carlos Elvira. Dr. Maria Nash provided the results of advancing contact angle measurements performed on the copolymers[178] (figure 25)

<table>
<thead>
<tr>
<th>NIPAM:EPM</th>
<th>LCST Onset (ºC)</th>
<th>LCST Midpoint (ºC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100:0</td>
<td>32.1</td>
<td>33.2</td>
</tr>
<tr>
<td>90:10</td>
<td>33.1</td>
<td>34.4</td>
</tr>
<tr>
<td>80:20</td>
<td>33.5</td>
<td>34.5</td>
</tr>
<tr>
<td>70:30</td>
<td>33.8</td>
<td>34.6</td>
</tr>
<tr>
<td>60:40</td>
<td>34.2</td>
<td>35.1</td>
</tr>
</tbody>
</table>

Table 3: LCST's of NIPAM/EPM copolymers
Figure 25: Increasing EPM content within the copolymers leads to an increase in LCST and decrease in contact angle indicating increasing hydrophilicity with increasing EPM content.

The fluorescence probes 4′-(diethylamino)-3-hydroxyflavone (FE), 5,6-benzo-4′-(diethylamino)-3-hydroxyflavone (BFE), and 4′-(diethylamino)-3-hydroxy-7-methoxyflavone (MFE) were obtained from Dr. Andrey S. Klymchenko, Laboratoire de Biophotonique et Pharmacologie, Université de Strasbourg, Faculté de Pharmacie, Illkirch, Cedex, France. These probes were synthesised as previously described [109, 153, 158], were used as received without any further purification and
their chemical structures are shown in figure 26. Reichardt’s betaine dye 2,6-diphenyl-4-(2,4,6 triphenylpyridinium-1-yl)phenolate was purchased from Sigma Aldrich and used as received. Its chemical structure is shown in figure 26.

![Chemical structures of probes used in this work.](image)

**Figure 26:** Chemical structures of probes used in this work.

### 2.2 Thin Film Preparation

Quartz slides of dimensions 12 mm x 45 mm x 1.5 mm (purchased from Lightpath Optical Ltd. UK) were used as the supporting substrates for the polymer films. Prior to film casting, the quartz slides were washed at least three times with acetone, methanol and deionized water and were dried in an oven at 70°C. The films were cast onto the quartz substrates yielding dry films of 10 μm in thickness using the following procedure: 5.4 mg of the solid polymer was dissolved in 137.5 μL of a 5.7 x 10\(^{-5}\) M ethanol (EtOH) solution of the requisite probe (~5wt% solution) (in the case of
Reichardt’s dye a 1 x 10^{-2} M solution of the probes was used as it was necessary to use a more concentrated EtOH solution of the probe. The solution was then carefully spread onto the quartz substrate and cured for 24 hours in a sealed EtOH environment. The EtOH was present to saturate the atmosphere above the polymer film to prevent water infiltration into the films during the curing process. On removal of the films from the EtOH environment they were placed in an oven at 70ºC for 48 hours to complete the drying process. By visual inspection the films obtained were smooth, transparent and free of any inhomogeneities. This thorough drying process is essential (especially the first stage of drying i.e. saturation of the environment with EtOH) because if moisture from the air (which may vary from day to day) absorbs onto the film, phase separation may occur which in turn will result in the film being rough and opaque which could lead to negative implications during analysis and also may lead to non-reproducible films. Schild [33, 47] explains that PNIPAm is subject to a phenomenon known as “cononsolvency” (meaning that it is soluble in both water and EtOH however it is insoluble in a mixture of water and EtOH). He explains that this effect is due to the preferential complexation of water and alcohol solvent over polymer-solvent complexation; in other words when EtOH and water are mixed (below the LCST) then the polymer precipitates or phase separates. Thus for this reason it is imperative that samples are prevented from interacting with the water present in the air during the drying process.

The film thickness was verified with a micrometer gauge. First the blank quartz substrate was measured and then post film casting and drying a minimum of 28 measurements were taken at various different locations across the sample. A film thickness of 10 µm was confirmed.

Average measured thickness of film + substrate (n=28) = 1.248 mm

Measured thickness of blank substrate = 1.238

Thickness of film = 0.01 mm = 10 µm
2.3 Instrumentation

A VGI2000M controlled humidity chamber (Surface Measurement Systems Ltd. UK) was used to generate a precisely controlled humidity and temperature environment in which the thin films were placed (operating under the principle of isothermal humidity generation). The chamber itself is designed to hold a standard recessed microscope slide and has double glazed windows on the top and bottom to allow for transmission or reflected illumination of the sample. The inside of the chamber consists of a stainless steel sample head which acts as a humidity generation reservoir and provides uniform temperature distribution around the sample. This is achieved by circulating water through a series of channels in the head using a thermoelectric heating and cooling stage to maintain the desired temperature. A precise humidity level is generated within the chamber by mixing water saturated nitrogen (N₂) gas with dry N₂ gas in the appropriate proportion under computer control. A miniature temperature and humidity sensor provides instant feedback of the temperature and humidity in the sample region. A schematic diagram depicting the generation of humidity within the chamber can be seen in figure 27.

Figure 27: (A) VGI200M controlled humidity chamber, (B) Schematic depicting generation of humidity within the chamber.
2.4 Spectroscopic Measurements

Both steady-state and time-resolved data were collected via the transparent glass window of the humidity chamber. In general, all tests began at 10% relative humidity (RH), and then humidity was cycled up in increments of 10%RH for steady-state measurements and in 20% RH increments for lifetime measurements. Each sample was analyzed in triplicate at 25º and 37/38ºC (below and above the LCST of the polymer systems). For steady-state measurements two different systems were utilised.

2.4.1 Steady-State Emission Measurements.

System A (commercial PNIPAm films): The excitation source was a fiber coupled modulated 405 nm laser diode (MDL 300, PicoQuant), and the detection system was a fiber optic coupled miniature spectrometer (USB2000 Ocean Optics). The excitation and emission fibers were coupled into a home-built assembly which had a dichroic beam-splitter to reflect the excitation light down into the chamber and then pass emission upward through a 430 nm long pass filter into a 1000 µm diameter optical fiber connected to the USB spectrometer (figure 28).

System B (poly(NIPAM-co-EPM films): The excitation source was a pulsed 405 nm laser diode (PicoQuant) and this was coupled into the central fiber of a fiber-optic reflection probe (R400-7 UV-vis, Ocean Optics) which has one illumination and six read fibers (all 400 µm diameter). The probe was used to couple the excitation light down into and fluorescence emission out of the humidity chamber. Emission was passed out of the chamber through a 430 nm long pass filter into a 1000 µm diameter optical fiber connected to a USB miniature spectrometer (USB2000, Ocean Optics) (figure 29).

The resulting steady-state spectra were not corrected for instrument response. Therefore caution should be exercised when comparing steady-state emission data collected with other published data; however for the purpose of this study with regard to changes due to humidity and probe structure we do not require the use of corrected spectra. The log(I_N*/I_T*) ratios and full width at half maximum (fwhm) values were
obtained using Origin (Origin version 7.0, Origin Lab Corporation, Northampton, MA). The spectra were first smoothed using the FFT function. The normalised integral intensity was calculated again using Origin 7 by means of the calculus-integrate function.

Figure 28: Picture and schematic diagram of system A. (1) Humidity Chamber (2) Dichroic beam-splitter (3) 430 nm long pass filter (4) 1000 µm fibre connected to USB spectrometer and (5) 400 µm fiber connected to 405 nm laser diode.

Figure 29: Picture and schematic diagram of system B. (1) Humidity Chamber (2) and (3) fiber optic reflection probe connected to 405 nm pulsed laser diode (for excitation) and passing emission through 430 nm long pass filter (4) 430 nm long pass filter (5) 1000 µm fiber connected to USB spectrometer.
2.4.2 Lifetime Measurements.

For the fluorescence lifetime measurements a fiber-optic reflection probe (R400-7, UV-vis, Ocean Optics) with one illumination and six read fibers (all 400 µm diameter) was used to couple the excitation light into and fluorescence emission out of the chamber. A pulsed 405 nm laser diode (PicoQuant) was used as the excitation source, and this was coupled into the central fiber of the optical probe. The fluorescence emission collected by the probe was coupled via a wedge depolarizer (WDPOL, Thorlabs) into an in-house assembled time-correlated single photon counting (TCSPC) system. This comprised of a monochromator (model 9030, Sciencetech, Canada) with a photon counting PMT detector (model H5783, Hamamatsu) (figure 30). Data collection was controlled by a PicoHarp 300 TCSPC system (PicoQuant). The instrument response function (IRF) was generated by collecting the scattered laser light from a clean quartz slide target which was placed inside the humidity chamber. Lifetimes were measured at emission wavelengths of 489 nm (N* maximum) and 564 nm (T*maximum) for FE, 487 nm and 587 nm for MFE, and at 502 nm and 563 nm for BFE. In each case data were collected until there were 10,000 counts in the channel of maximum intensity, and then fluorescence lifetimes were extracted from the measured decay curves using FluoFit (version 4.2, PicoQuant) which implements nonlinear least-squares error minimization analysis, based on the Simplex and Lavenberg-Marquardt algorithms. The final quoted result was determined by the fit, which had a $\chi^2$ value of less than 1.2 and a residual trace that was symmetric about zero. The fiber-optic-based sampling arrangements yielded a spot size of ~5 mm² for all the spectroscopic measurements undertaken.
Figure 30: Picture of the Lifetime system.

2.5 UV-visible study: NIPAM-EPM thin films doped with Reichardt’s dye.

A tungsten halogen lamp (LS-1, Ocean Optics) was used as the light source for UV-vis measurements. Excitation light was coupled into the chamber by means of a 1000 µm diameter optical fiber via a 3.3ND filter. Light was passed out of the chamber through a 600 µm diameter optical fiber which was connected to the USB miniature spectrometer (figure 31). Reference measurements were taken by placing a clean quartz slide inside the chamber.

Figure 31: Picture and schematic diagram of system used for UV-visible study. (1) Humidity Chamber (2) 3.3 ND filter (3) 600 µm optical fiber connected to USB spectrometer(4) 1000 µm fiber connected (5) Tungsten halogen lamp.
Measurements were performed at 25°C (below the LCST’s) and relative humidity (RH) was cycled up in increments of 20%RH from 10%RH to 90%RH. Measurements were taken every 30 minutes and each sample was analysed in triplicate.

3.1 Introduction

Previously 3-hydroxyflavone (3-HF) probes were utilised to assess the polarity of poly(NIPAM-co-NtBA) copolymer films.[76, 88] During the course of this research it was noted that moisture uptake by the copolymer films resulted in significant emission changes. It was hypothesised that water incorporated into the polymer film led to a more equilibrated ESIPT process, suggesting that 3-HF probes may be useful fluorescence sensors for assessing water uptake within polymer films.[88] Here we analyse the water uptake in thin PNIPAm films supported on quartz substrates by measuring the fluorescence emission properties of a series of 3-HF fluorophores (FE, MFE and BFE). This is done to determine which fluorophore is the most sensitive to water uptake and to study in detail the dynamic hydration processes in thin thermoresponsive polymer films.

3.2 Equilibration

The measurement of emission data for FE, BFE and MFE in liquid solvents and correlation with physicochemical properties of the solvent is, for the most part, straightforward due to the homogeneous local environment of the solvent (a linear correlation of log(I_N*/I_T*) values with solvent polarity was observed).[109, 153, 158] In the case of polymer films however it is more complex. It has been previously shown that while these fluorophores can be used to readily measure changes in the chemical composition of dry hydrophilic/hydrophobic thermoresponsive copolymer films, the quantitative measurement of polarity however proved to be more difficult. This is due to heterogeneity in the ground state hydrogen bonding which was evidenced by an excitation wavelength dependence for the N* and T* band emission.[76, 88]

An in-depth study was first conducted to determine the equilibration times...
required for reproducible measurements from the polymer thin films (i.e. the time taken to extract sufficient water out of the chamber environment and diffuse throughout the polymer and establish an equilibrium). Replicate measurements made on a thin film cast from a 4% PNIPAm solution, with short equilibration times (10 minutes ascending and 15 minutes descending), show poor reproducibility (figure 33).

There is considerable hysteresis in the plot of log(I_N*/I_T*) versus %RH when the cycle of measurements went from low (10%RH) to high (90%RH) and back to low (10%RH) (figure 32). A method was developed for solvents to measure the polarity and hydrogen bonding using the log of intensity ratio between the N* and T* bands [109, 153, 158], and so by extension of this method log(I_N*/I_T*) values were used as these values provide the best indication of changes in the local environment due to water sorption.

Figure 32: Plot of log(I_N*/I_T*) versus relative humidity for PNIPAm doped with FE showing hysteresis. On ascending humidity measurements were taken every 10 minutes and on descending humidity measurements were taken every 15 minutes. Error bars represent the standard deviation (S.D.) from the average of 3 replicate measurements. S.D. at 10% = ± 0.0007 (ascending RH) and ± 0.009 (descending RH) S.D. at 90% = ± 0.008 (ascending RH) and ± 0.0113 (descending RH).
The equilibration time is obviously a function of polymer composition and water content level, and it was found (through lengthy trial and error experimentation) that for PNIPAm thin films a minimum one hour equilibration period was required for every 10% change in relative humidity. Figure 33 below displays an example of one equilibration study undertaken to determine the amount of time required before a reproducible measurement can be made. Measurements were taken every 15 minutes at a constant relative humidity level (30% in this case) and temperature (25°C) and as can be seen from the plot the sample appears to have equilibrated after ~60 minutes (after 60 minutes only a very small fluctuation in log(I_N*/I_T*) values of 0.001 is observed).

![Figure 33: Plot of log(I_N*/I_T*) versus time of a PNIPAm film doped with FE. A constant relative humidity was set at 30% and measurements were taken every 15 minutes.](image)

Another important factor in achieving good reproducibility and equilibration was film drying prior to placement in the chamber. It was found (again through trial and error experimentation) that samples required 84 hours drying time in an oven at 70°C between replicate measurements. Figure 34 shows results obtained from a PNIPAm sample doped with FE. The sample was analysed at 25°C from 10%RH to
90%RH in 10% increments at hourly intervals. Once 90%RH was reached the sample was removed from the chamber and placed in an oven at 70°C for 48 hours before repeating the measurement. As can be seen from the plot below the data is not very reproducible suggesting that the sample was not given sufficient drying time and there still may be some residual water present in the polymer matrix.

Figure 34: Plot of log($I_{N^-}/I_{T^+}$) versus relative humidity for a PNIPAm sample doped with FE. Measurements were made at 25°C and a 48 hour drying time was used between replicate measurements. Error bars represent the standard deviation (S.D.) from the average of 3 replicate measurements. S.D at 10% = ± 0.009 and S.D. at 90% = ± 0.0134.

After several trials, we found that a drying time of ~84 hours gave sufficient reproducibility (figure 35).
Figure 35: Plot of $\log(I_{N^*}/I_{T^*})$ versus relative humidity for a PNIPAm sample doped with FE. Measurements were made at 25°C and a drying time of 84 hours was used between replicate measurements on the same film. Error bars represent the standard deviation (S.D.) from the average of 3 replicate measurements. S.D. at 10% = ± 0.012 and S.D. at 90% = ± 0.004.

It was also noted from replicate measurements that the standard deviation in the measurements is larger at lower relative humidities and this may be expected as the dry polymers have a significant water uptake capacity. At low RH the amount of available water present is low; therefore it will take longer to extract water out of the chamber environment and diffuse throughout the polymer before establishing an equilibrium.

### 3.3 Steady-State Fluorescence Emission Spectroscopy

At a temperature of 25°C (below the LCST), as the relative humidity is raised and water is adsorbed by the polymer film there is a wavelength shift for both N* and T* emission bands; the N* band is shifted to the red by up to 10 nm while the T* band emission is blue-shifted by a much smaller amount (~2 nm). This trend was observed for each of the three fluorophores. The normalised fluorescence emission spectra for PNIPAm films doped with FE, BFE and MFE are shown in figure 36 (to facilitate easier comparison of the data presented the spectra shown have been normalized to
the maximum of the $N^\ast$ band).

Figure 36: Smoothed normalized (to $N^\ast$ band) emission spectra of (A) FE, (B) BFE and (C) MFE in PNIPAm collected at different relative humidity (between 10% and 90%).

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1 The distortion in the emission spectra visible below ~450 nm is due to the cutoff filter used to eliminate scattered light.
The large observed N*-T* band separation arises from the presence of a significant polymer-probe H-bonding interaction[76], a similar process has been observed in protic solvents.[109]

There are two stages of wavelength shifts observed for the N* band: first, a small change (< 2 nm) below ~40%RH and then a more dramatic variation at higher water content (figure 37). With increasing humidity the specific interaction of the fluorophores with the polymer is interrupted (i.e. the specific H-bonding interaction between the polymer and probes is interrupted by incoming water molecules). This interruption, due to the hydration of the amide hydrogen and/or carbonyl group of the polymer, enhances intramolecular hydrogen bonding and increases dielectric stabilisation resulting in the red-shift of the N* band.
Figure 37: Plot of variation in N* and T* band positions against increasing %RH for PNIPAm doped with (A) FE, (B) BFE and (C) MFE.

Accompanying the wavelength shift is a variation in intensity at the maximum of both the N* and T* bands (figure 38)
Figure 38: Plot showing variation of intensity at the maximum of N* and T* emission bands for PNIPAm films doped with (A) FE, (B) BFE and (C) MFE.
Table 4: Summary of the total N*-T* band separation at 10% and 90% RH for PNIPAm films doped with FE, BFE and MFE.

<table>
<thead>
<tr>
<th></th>
<th>ΔN*-T* separation 10% RH</th>
<th>ΔN*-T* separation 90% RH</th>
</tr>
</thead>
<tbody>
<tr>
<td>FE</td>
<td>~ 75 nm</td>
<td>~ 62 nm</td>
</tr>
<tr>
<td>BFE</td>
<td>~ 60 nm</td>
<td>~ 52 nm</td>
</tr>
<tr>
<td>MFE</td>
<td>~ 79 nm</td>
<td>~ 66 nm</td>
</tr>
</tbody>
</table>

We first observed a small increase of N* band intensity for FE and MFE with increasing humidity, however above approximately 50%RH a decrease in intensity is noted. A similar observation to that of the N* band intensity is seen in the case of T* band intensity; FE and MFE display a slight increase in intensity up to ~ 50%RH followed by a relatively dramatic decrease in band intensity at higher relative humidities. BFE displays a gradual decrease across all humidity levels.

As humidity increases, the overall fluorescence intensity decreases, as can be seen from the response of the integrated spectral intensity\(^2\) (figure 39) and the same trend was observed for all three fluorophores. For FE and MFE the overall intensity change seems to follow two distinct phases: first, a small increase up ~50%RH, followed by a much steeper decrease at higher relative humidity. For BFE, the intensity changes are more pronounced, and a large decrease at low humidity is observed followed by a much steeper decrease above ~60%RH. This intensity decrease at higher relative humidity may be due to water induced static quenching\(^3\). The two-stage behaviour of both wavelength shifts and intensity variations indicate that the degree of N* state dielectric stabilization is greater when there is more water present.

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\(^2\) The area under the curve from 425nm-600nm: calculated in Origin ver. 7.0 by means of the calculus-integrate function.

\(^3\) Refer to lifetime data in section 1.4
Figure 39: Plot of normalised integral intensity versus relative humidity for thin PNIPAm films doped with FE, BFE and MFE.

The greater error in the BFE measurements is due to difficulties in getting reproducible replicate measurements, which arose largely from the much greater photobleaching encountered with this fluorophore (figure 40). This photobleaching may also account for the more pronounced decrease of integral intensity with increasing RH.
Figure 40: Fluorescence intensity versus time plots, showing the different photobleaching rates of the three 3-HF fluorophores in thin polymer films for N* band emission (A) and T* band emission (B). This data was collected from the probes doped into a 65:35 poly(N-isopropylacrylamide-N-tert- butylacrylamide) (NIPAm-co-NtBA) copolymer which was then cast into a thin film. These copolymers have very similar characteristics to PNIPAm.[76]

The photobleaching measurements were made under nearly identical conditions as per PNIPAm thin films; measurements began at 10%RH and progressed to 90%RH in 10% increments in one hour intervals and intensity measurements were taken every fifteen minutes. It is clear that the photobleaching of the BFE fluorophore is much more significant than for the other two probes (FE and MFE).

3.3.1 Bandwidths (Full Width at Half Maximum, fwhm)

Computational analysis of the normalised emission spectra shows that there are significant changes in the full width at half maximum (fwhm) of both emission bands (figure 41).
Figure 41: (A) Plot of N* bandwidths (full widths at half maximum, nm) for all three probes versus increasing humidity. (B) Plot of T* bandwidths for all three probes versus increasing humidity.

The peak width of the emission bands of these probes provides information about the changes in the microheterogeneity of the polymer matrix as water is adsorbed. The wider the band becomes indicates an increase in heterogeneity. We see big differences between FE/MFE on one hand and BFE on the other. For the N* band of FE and MFE, as humidity is increased there is a decrease in fwhm of ~ 10 nm, indicating that the fluorophores are now experiencing a narrower range of microenvironments as water is absorbed (figure 41 A). For BFE the situation is reversed, and at higher relative humidity (> 60%) the bandwidth increases slightly (by ~ 3 nm), indicating that the N* state is experiencing a slightly more homogeneous environment. In contrast, the T* bandwidth seems to decrease slowly (~ 1.5 nm) for FE and MFE at relative humidity below ~ 80% but then decreases more rapidly above ~ 80% RH. For BFE, there is a slightly larger decrease in fwhm of ~ 3 nm at higher relative humidity (>50%RH) which may indicate a more homogeneous T* environment.

3.3.2 Intensity Ratio Parameter.

Plotting the log of intensity ratio (log(I_{N*}/I_{T*})) versus relative humidity for all three fluorophores (figure 42 (A)), it can be seen that on increasing the relative
humidity, $\log(I_{N^*}/I_{T^*})$ values also increase, but this occurs at two different rates with the greatest increase occurring at the higher relative humidity’s above ~ 50% RH. This may be attributed to a dramatic increase in local polarity due to water absorption, which in turn causes a greater relative dielectric stabilization of the N* state compared to the T* state, leading to an increase in the intensity of the N* emission relative to the T* emission.[109]

![Graphs](image.png)

**Figure 42:** (A) Plot of the $\log(I_{N^*}/I_{T^*})$ ratio versus increasing relative humidity at 25°C for PNIPAm films doped with FE, BFE and MFE and (B) Plot of the $I_{N^*}/I_{T^*}$ ratio normalized to the value recorded at 90%RH.

In the case of MFE at lower RH, the weak methoxy electron donor at the 7-position decreases the charge transfer character of the N* state, making the N* state dielectrically less stabilized, which favours the ESIPT product tautomer (T*) state, giving a lower $\log(I_{N^*}/I_{T^*})$ ratio across all humidity values (figure 42). In contrast to FE and MFE, BFE should be less sensitive to hydrogen bonding with 4-carbonyl because of its steric hindrance caused by the additional benzyl group. This is evidenced by the lower $\Delta N^*-T^*$ band separation values observed for BFE (lower values indicate a lesser degree of H-bonding interaction between BFE and the polymer) compared to FE and MFE (Table 4).

Interestingly, BFE is more sensitive to humidity changes than FE and in fact has an almost identical sensitivity to relative humidity as MFE (figure 42). This therefore
suggests that it is the specific interaction between the 3 hydroxy group of the fluorophore and the carbonyl group of the polymer which is responsible for the observed large N*-T* separation in the dry state and at low relative humidity.[76, 88] It could be explained that the humidity mainly affects the polarity of the polymer environment, but not its H-bond donor ability, which is in line with the observed red shifts of the N* emission bands for all three fluorophores on the addition of water at low relative humidity (figure 37). Thus the additional water molecules are more than likely strongly H-bonded to the carbonyl oxygen of PNIPAm, which neutralises the high H-bond donor ability of water and explains the absence of the specific H-bonding effects with the FE and MFE fluorophores.

To more readily observe the magnitude of the water-induced changes, they have been compared by plotting the normalized values of the I_N*/I_T* ratios versus increasing relative humidity (figure 42 (B)). Both BFE and MFE display the largest relative changes whereas there is virtually no significant change in the case of FE at lower RH. The significantly larger changes in the log(I_N*/I_T*) values as a function of increasing humidity for MFE, together with its relatively good photostability, makes it the best probe for studying water uptake dynamics in these thin polymer films.

It is clear that these 3-HF fluorophores sense two distinct physicochemical environments as the humidity increases and more water is absorbed by the polymer film. All the observed changes in emission data may be explained by variations in hydrogen-bonding interactions. These interactions have been previously observed in PNIPAm and NIPAM-NtBA copolymers.[76] Based on prior solvatochromic measurements [21] which showed that PNIPAm was a strong hydrogen-bond acceptor ($\beta = \sim 0.76 \pm 0.03$) and a moderately strong hydrogen-bond donor ($\alpha = \sim 0.40 \pm 0.03$), it is obvious that PNIPAm under these water uptake conditions is a relatively strong protic environment. The FE probe has an extremely low quantum yield in water (< 0.3%) but a relatively high quantum yield in protic solvents which are strong H-bond acids. Therefore, the absence of the quenching of the fluorophores at low relative humidity is probably due to the water molecules being strongly associated with the polymer.
In this relatively dry domain, the incoming water will preferentially locate in the hydrophilic polymer domains where the most polar groups of the polymer are located. We suggest that this incoming water will first hydrogen bond strongly with the polymer N-H and C=O groups and therefore will not solvate the fluorophore. This argument is supported by molecular dynamics studies of water interactions with PNIPAm by Tamai [179, 180] which indicates that water molecules are strongly bound to the N-H and C=O of the polymer at relatively low water content. Thus, the log(I_N*/I_T*) value for FE (figure 42) is relatively constant at these lower water levels because this polymer-bound water does not have a very significant impact on the ESIPT process.

In the second phase of hydration (> 50% RH), where there is a greater adsorbed water content, the incoming water appears to preferentially associate with the water bound to the polymer (the primary hydration shell), generating larger loosely bound water domains within the polymer. The amount of adsorbed water is potentially considerable, given that at a relative humidity of 90% and at a temperature of 20°C (below PNIPAm's LCST), bulk PNIPAm is capable of absorbing a maximum of ~8% of its own dry weight in water.[181] At this stage, for all three fluorophores, we see dramatic changes in the log(I_N*/I_T*) ratio values (figure 42), greater red shift of the N* emission band position (figure 37), decreases in fluorescence integral intensity (figure 39), but we do not see any significant change in the local heterogeneity experienced by the excited states (figure 41) (full width half maximum decreases for the N* state thus indicating a reduction in heterogeneity of the emitting states).

It can be suggested then that this second phase hydration produces spectral effects similar to those produced by the common solvent polarity effect with increasing dielectric stabilisation of the N* state, causing a greater red shift of the N* band and a large relative increase in log(I_N*/I_T*) ratio.[174] These effects are very different from those observed from the hydration of AOT reverse micelles.[174] In this study on the hydration of AOT reverse micelles it is reported that the addition of water results in an increased ordering of the AOT molecules and the redistribution of the FE fluorophore into the apolar hexane phase, ultimately resulting in the decrease of log(I_N*/I_T*) ratio.
values. This trend is not observed with PNIPAm because there is no distinct apolar phase and there is no clear evidence for redistribution of fluorophore into more hydrophilic microdomains.

3.3.3 Measurements Above and Below the LCST.

Above the LCST (which occurs at 32°C) PNIPAm exists in a more hydrophobic, condensed state and therefore the rate of water absorption should be decreased. Previous reports using thermal gravimetric analysis on PNIPAm [181] indicated that no water was absorbed at 40°C (or to be more precise, it was not possible to measure the low amount of water absorbed). However this is not the case with thin films of this PNIPAm polymer at 37°C. Fluorescence analysis of the thin PNIPAm films show clearly that in every case the log(I_N/I_T) increases with increasing humidity (figure 43).
Figure 43: Plot of the log($I_N$/$I_T$) ratio versus humidity of PNIPAm thin films doped with (A) FE, (B) BFE and (C) MFE at 25°C and 37°C.

This provides an indication that water has indeed infiltrated the polymer film above the LCST, interrupting polymer-fluorophore interaction. This is not altogether surprising since the much larger surface area of the thin film will encourage adsorption of water. The log($I_N$/$I_T$) ratio values for the measurements performed at
37°C are in all cases lower than what was observed for the 25°C measurements. This clearly indicates the less polar environment of the condensed polymer state above the LCST. As water infiltrates the polymer, the polymer-fluorophore interactions are supplanted by (polymer-water)-fluorophore interactions, leading to an increasing stabilization of the N* state relative to the T* state, and a similar dependence of \( \log(I_{N*}/I_{T*}) \) versus humidity to that observed at 25°C is noted. This then indicates that in thin micrometre scale polymer films, even above the LCST of the polymer, water adsorption could still be a significant issue.

### 3.4 Time Resolved Fluorescence Measurements

In order to try and understand the photophysical environment in more detail, fluorescence lifetime measurements were performed. Lifetime data was collected for both emission bands of all three fluorophores doped into PNIPAm thin films under conditions of varying humidity (the same as described for steady-state measurements although humidity was increased in increments of 20%RH for lifetime data collection and measurements were performed below the LCST only, at 25°C).

Each lifetime measurement took approximately between 10 and 15 minutes to complete (until there was 10,000 counts in the channel of maximum intensity). Data collection took longer at higher water content. We can see (figures 44, 45 & 46, tables 5, 6 & 7) that all recorded decays were triexponential, consisting of a fast decay component at the emission of both the N* and T* bands. \( \tau_2 \) and \( \tau_3 \) values of the T* band are longer than the corresponding N* decay components thus suggesting that the ESIPT process is irreversible.[137, 182] The most significant differences between the N* and T* decay components were noted in the case of FE and MFE. FE displays a difference of between ~0.03 - 0.12, ~0.84 - 1.28 and ~0.97 – 1.30 between the N* and T* states of \( \tau_1 \), \( \tau_2 \) and \( \tau_3 \) respectively. MFE shows a difference of ~0.04 – 0.30, ~1.17 – 1.39 and ~1.12 – 3.05 for \( \tau_1 \), \( \tau_2 \) and \( \tau_3 \) respectively. In contrast the decay components of the T* band of BFE are close in value to those of the N* band showing a difference of ~0.05 – 0.27, ~0.05 - 0.51 and ~0.08 – 0.30 for \( \tau_1 \), \( \tau_2 \) and \( \tau_3 \) respectively. We
suggest that this is due to the additional aromatic ring in the BFE structure reducing the H-bonding ability at the carbonyl group.[158] The three decay components of the PNIPAm films doped with FE, MFE and BFE may be assigned to the H-bond free form, the H-bonded form and the ESIPT process, according to previous studies undertaken in phosphatidylglycerol vesicles and in solvents.[119, 168, 169]

Increasing RH leads to an increase in N* average lifetime ($\tau_{AV}$) and a decrease in T* $\tau_{AV}$ ($\Delta \tau_{AV}$ change N* 0.05, 0.12 and 0.04 ns for FE, BFE and MFE respectively, $\Delta \tau_{AV}$ change T* 0.19, 0.01 and 0.26 ns for FE, BFE and MFE respectively). Also noted was the greater magnitude of the T* value compared to N*. This trend was observed for all three probes. This decrease of the T* lifetime indicates that on increasing RH the probes sense a more solvent-like environment and the ESIPT process becomes more equilibrated.[88] This T* lifetime observation is supported by the observed decrease in N*-T* band separation (observed in steady-state data) with increasing water content (figure 37 and table 4).
<table>
<thead>
<tr>
<th>Relative Humidity (%)</th>
<th>Band</th>
<th>$\tau_1$ (ns)</th>
<th>$\tau_2$ (ns)</th>
<th>$\tau_3$ (ns)</th>
<th>$\alpha_1$</th>
<th>$\alpha_2$</th>
<th>$\alpha_3$</th>
<th>$\tau_{av}$ (ns)</th>
<th>$\chi^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 N*</td>
<td>0.25</td>
<td>1.35</td>
<td>2.51</td>
<td>0.23</td>
<td>0.25</td>
<td>0.52</td>
<td>2.22</td>
<td>0.99</td>
<td></td>
</tr>
<tr>
<td></td>
<td>± 0.035</td>
<td>± 0.17</td>
<td>± 0.045</td>
<td>± 0.092</td>
<td>± 0.104</td>
<td>± 0.519</td>
<td></td>
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<tr>
<td>10 T*</td>
<td>0.13</td>
<td>2.19</td>
<td>3.48</td>
<td>-0.29</td>
<td>0.29</td>
<td>0.42</td>
<td>3.14</td>
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<tr>
<td></td>
<td>± 0.017</td>
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<td>0.25</td>
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<tr>
<td></td>
<td>± 0.049</td>
<td>± 0.138</td>
<td>± 0.034</td>
<td>± 0.118</td>
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<td>± 0.017</td>
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<td>± 0.189</td>
<td>± 0.042</td>
<td>± 0.112</td>
<td>± 0.113</td>
<td>± 0.564</td>
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<tr>
<td>50 T*</td>
<td>0.20</td>
<td>2.52</td>
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<td>0.56</td>
<td>0.25</td>
<td>3.09</td>
<td>1.05</td>
<td></td>
</tr>
<tr>
<td></td>
<td>± 0.029</td>
<td>± 0.211</td>
<td>± 0.558</td>
<td>± 0.075</td>
<td>± 0.446</td>
<td>± 0.217</td>
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<tr>
<td>70 N*</td>
<td>0.26</td>
<td>1.20</td>
<td>2.56</td>
<td>0.24</td>
<td>0.22</td>
<td>0.54</td>
<td>2.27</td>
<td>0.96</td>
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<tr>
<td></td>
<td>± 0.042</td>
<td>± 0.137</td>
<td>± 0.031</td>
<td>± 0.089</td>
<td>± 0.073</td>
<td>± 0.399</td>
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<tr>
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<td>0.5</td>
<td>0.24</td>
<td>3.03</td>
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<tr>
<td></td>
<td>± 0.022</td>
<td>± 0.272</td>
<td>± 0.623</td>
<td>± 0.135</td>
<td>± 0.408</td>
<td>± 0.228</td>
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<tr>
<td>90 N*</td>
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<td>1.16</td>
<td>2.57</td>
<td>0.25</td>
<td>0.22</td>
<td>0.53</td>
<td>2.27</td>
<td>0.99</td>
<td></td>
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<tr>
<td></td>
<td>± 0.035</td>
<td>± 0.115</td>
<td>± 0.028</td>
<td>± 0.085</td>
<td>± 0.068</td>
<td>± 0.375</td>
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</tr>
<tr>
<td>90 T*</td>
<td>0.34</td>
<td>2.22</td>
<td>3.22</td>
<td>-0.22</td>
<td>0.35</td>
<td>0.44</td>
<td>2.95</td>
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<tr>
<td></td>
<td>± 0.035</td>
<td>± 0.49</td>
<td>± 0.369</td>
<td>± 0.056</td>
<td>± 0.281</td>
<td>± 0.429</td>
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</table>

Table 5: Fluorescence lifetime data for different relative humidities at 25°C for PNIPAm films doped with FE. All data was fitted to a triexponential model. N* data was measured at 490 nm and T* data was measured at 590 nm. $\tau_{av}$ = intensity averaged lifetime, $\alpha_i$ = fractional amplitudes/pre-exponential factors. Amplitudes have been normalized such that $\sum |\alpha_i| = 1$. 

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Figure 44: Plot of (A) N* average lifetime, (B) T* average lifetime, (C) N* lifetimes, (D) T* lifetimes, (E) N* fractional amplitude and (F) T* fractional amplitude, all versus increasing humidity for a PNIPAm thin film doped with FE. Errors calculated using support plane analysis.
<table>
<thead>
<tr>
<th>Relative Humidity (%)</th>
<th>Band</th>
<th>$\tau_1$ (ns)</th>
<th>$\tau_2$ (ns)</th>
<th>$\tau_3$ (ns)</th>
<th>$\alpha_1$</th>
<th>$\alpha_2$</th>
<th>$\alpha_3$</th>
<th>$\tau_{av}$ (ns)</th>
<th>$\chi^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 N*</td>
<td></td>
<td>0.01</td>
<td>1.26</td>
<td>2.96</td>
<td>0.21</td>
<td>0.16</td>
<td>0.63</td>
<td>2.79</td>
<td>1.09</td>
</tr>
<tr>
<td></td>
<td></td>
<td>± 0.022</td>
<td>± 0.084</td>
<td>± 0.021</td>
<td>-</td>
<td>± 0.065</td>
<td>± 0.103</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 T*</td>
<td></td>
<td>0.06</td>
<td>1.67</td>
<td>3.26</td>
<td>-0.42</td>
<td>0.10</td>
<td>0.48</td>
<td>3.15</td>
<td>0.98</td>
</tr>
<tr>
<td></td>
<td></td>
<td>± 0.007</td>
<td>± 0.044</td>
<td>± 0.215</td>
<td>± 0.357</td>
<td>± 0.249</td>
<td>± 0.056</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30 N*</td>
<td></td>
<td>0.01</td>
<td>1.26</td>
<td>2.99</td>
<td>0.29</td>
<td>0.15</td>
<td>0.56</td>
<td>2.81</td>
<td>1.02</td>
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<tr>
<td></td>
<td></td>
<td>± 0.07</td>
<td>± 0.092</td>
<td>± 0.023</td>
<td>-</td>
<td>± 0.064</td>
<td>± 0.106</td>
<td></td>
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</tr>
<tr>
<td>30 T*</td>
<td></td>
<td>0.12</td>
<td>1.55</td>
<td>3.22</td>
<td>-0.12</td>
<td>0.12</td>
<td>0.76</td>
<td>3.12</td>
<td>1.07</td>
</tr>
<tr>
<td></td>
<td></td>
<td>± 0.069</td>
<td>± 0.299</td>
<td>± 0.041</td>
<td>± 0.09</td>
<td>± 0.065</td>
<td>± 0.67</td>
<td></td>
<td></td>
</tr>
<tr>
<td>50 N*</td>
<td></td>
<td>0.01</td>
<td>1.04</td>
<td>2.99</td>
<td>0.50</td>
<td>0.10</td>
<td>0.40</td>
<td>2.83</td>
<td>1.13</td>
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<tr>
<td></td>
<td></td>
<td>± 0.232</td>
<td>± 0.073</td>
<td>± 0.017</td>
<td>-</td>
<td>± 0.044</td>
<td>± 0.065</td>
<td></td>
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</tr>
<tr>
<td>50 T*</td>
<td></td>
<td>0.07</td>
<td>1.15</td>
<td>3.15</td>
<td>-0.44</td>
<td>0.06</td>
<td>0.50</td>
<td>3.13</td>
<td>1.07</td>
</tr>
<tr>
<td></td>
<td></td>
<td>± 0.006</td>
<td>± 0.019</td>
<td>± 0.117</td>
<td>± 0.252</td>
<td>± 0.136</td>
<td>± 0.023</td>
<td></td>
<td></td>
</tr>
<tr>
<td>70 N*</td>
<td></td>
<td>0.11</td>
<td>1.45</td>
<td>3.14</td>
<td>0.27</td>
<td>0.17</td>
<td>0.56</td>
<td>2.89</td>
<td>0.94</td>
</tr>
<tr>
<td></td>
<td></td>
<td>± 0.077</td>
<td>± 0.747</td>
<td>± 0.396</td>
<td>± 0.155</td>
<td>± 0.053</td>
<td>± 0.233</td>
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<tr>
<td>70 T*</td>
<td></td>
<td>0.21</td>
<td>1.50</td>
<td>3.23</td>
<td>-0.10</td>
<td>0.12</td>
<td>0.78</td>
<td>3.13</td>
<td>0.91</td>
</tr>
<tr>
<td></td>
<td></td>
<td>± 0.098</td>
<td>± 0.34</td>
<td>± 0.04</td>
<td>± 0.069</td>
<td>± 0.062</td>
<td>± 0.674</td>
<td></td>
<td></td>
</tr>
<tr>
<td>90 N*</td>
<td></td>
<td>0.30</td>
<td>1.24</td>
<td>3.13</td>
<td>0.15</td>
<td>0.17</td>
<td>0.68</td>
<td>2.91</td>
<td>1.16</td>
</tr>
<tr>
<td></td>
<td></td>
<td>± 0.085</td>
<td>± 0.198</td>
<td>± 0.032</td>
<td>± 0.116</td>
<td>± 0.120</td>
<td>± 0.644</td>
<td></td>
<td></td>
</tr>
<tr>
<td>90 T*</td>
<td></td>
<td>0.57</td>
<td>0.92</td>
<td>3.21</td>
<td>-0.16</td>
<td>0.17</td>
<td>0.67</td>
<td>3.16</td>
<td>1.03</td>
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<tr>
<td></td>
<td></td>
<td>± 0.543</td>
<td>± 0.791</td>
<td>± 0.026</td>
<td>± 0.132</td>
<td>± 0.126</td>
<td>± 0.578</td>
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<td></td>
</tr>
</tbody>
</table>

Table 6: Fluorescence lifetime data for different relative humidities at 25°C for PNIPAm films doped with BFE. All data was fitted to a triexponential model. N* data was measured at 502 nm and T* data was measured at 590 nm. $\tau_{av}$ = intensity averaged lifetime, $\alpha_i$ = fractional amplitudes/pre-exponential factors. Amplitudes have been normalized such that $\sum |\alpha_i| = 1.$
Figure 45: Plot of (A) N* average lifetime, (B) T* average lifetime, (C) N* lifetimes, (D) T* lifetimes, (E) N* fractional amplitude and (F) T* fractional amplitudes, all versus increasing humidity for a PNIPAm thin film doped with BFE. Errors calculated using support plane analysis.
Relative Humidity (%) & Band & $\tau_1$ (ns) & $\tau_2$ (ns) & $\tau_3$ (ns) & $\alpha_1$ & $\alpha_2$ & $\alpha_3$ & $\tau_{av}$ (ns) & $\chi^2$
\hline
10 & N* & 0.41 & 1.51 & 2.52 & 0.20 & 0.35 & 0.45 & 2.11 & 1.09 \\
& & $\pm 0.066$ & $\pm 0.228$ & $\pm 0.111$ & $\pm 0.121$ & $\pm 0.347$ & $\pm 0.442$ & \\
10 & T* & 0.18 & 2.84 & 4.31 & -0.30 & 0.50 & 0.20 & 3.48 & 1.04 \\
& & $\pm 0.015$ & $\pm 0.271$ & $\pm 0.84$ & $\pm 0.208$ & $\pm 0.413$ & $\pm 0.187$ & \\
30 & N* & 0.55 & 1.86 & 2.67 & 0.25 & 0.45 & 0.30 & 2.13 & 1.15 \\
& & $\pm 0.072$ & $\pm 0.459$ & $\pm 0.504$ & $\pm 0.277$ & $\pm 0.441$ & $\pm 0.303$ & \\
30 & T* & 0.20 & 3.03 & 5.72 & -0.28 & 0.66 & 0.06 & 3.49 & 0.98 \\
& & $\pm 0.016$ & $\pm 0.105$ & $\pm 2.056$ & $\pm 0.208$ & $\pm 0.413$ & $\pm 0.187$ & \\
50 & N* & 0.53 & 1.70 & 2.64 & 0.25 & 0.37 & 0.38 & 2.15 & 1.17 \\
& & $\pm 0.071$ & $\pm 0.397$ & $\pm 0.25$ & $\pm 0.198$ & $\pm 0.317$ & $\pm 0.381$ & \\
50 & T* & 0.24 & 2.90 & 4.76 & -0.26 & 0.63 & 0.11 & 3.39 & 1.01 \\
& & $\pm 0.021$ & $\pm 0.177$ & $\pm 1.413$ & $\pm 0.117$ & $\pm 0.531$ & $\pm 0.096$ & \\
70 & N* & 0.34 & 1.46 & 2.62 & 0.28 & 0.32 & 0.40 & 2.15 & 1.10 \\
& & $\pm 0.039$ & $\pm 0.164$ & $\pm 0.085$ & $\pm 0.086$ & $\pm 0.264$ & $\pm 0.346$ & \\
70 & T* & 0.27 & 2.79 & 3.74 & -0.24 & 0.51 & 0.25 & 3.25 & 1.04 \\
& & $\pm 0.025$ & $\pm 0.741$ & $\pm 1.773$ & $\pm 0.084$ & $\pm 0.501$ & $\pm 0.244$ & \\
90 & N* & 0.32 & 1.43 & 2.66 & 0.30 & 0.32 & 0.38 & 2.15 & 1.05 \\
& & $\pm 0.03$ & $\pm 0.129$ & $\pm 0.07$ & $\pm 0.075$ & $\pm 0.209$ & $\pm 0.285$ & \\
90 & T* & 0.28 & 2.82 & 4.19 & -0.29 & 0.60 & 0.11 & 3.22 & 1.04 \\
& & $\pm 0.017$ & $\pm 0.279$ & $\pm 2.021$ & $\pm 0.082$ & $\pm 0.559$ & $\pm 0.105$ & \\
\hline

Table 7: Fluorescence lifetime data for different relative humidities at 25°C for PNIPAm films doped with MFE. All data was fitted to a triexponential model. N* data was measured at 487 nm and T* data was measured at 590 nm. $\tau_{av}$ = intensity averaged lifetime, $\alpha_i$ = fractional amplitudes/pre-exponential factors. Amplitudes have been normalized such that $\sum|\alpha_i|=1$. 

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Figure 46: Plot of (A) N* average lifetime, (B) T* average lifetime, (C) N* lifetimes, (D) T* lifetimes, (E) N* fractional amplitude and (F) T* fractional amplitudes, all versus increasing humidity for a PNIPAm thin film doped with MFE. Errors calculated using support plane analysis.

For FE, BFE and MFE the fractional amplitudes do not vary much in the relative humidity range sampled. Normally, FE derivatives in organic solvents show two decay times for both the N* and T* bands. Also, due to the reversible ESIPT reaction of FE in organic solvents the following observations have been reported [169]: (1) a
short decay time for the N* band corresponding to the ESIPT reaction, whereas for the T* band, the same decay time is associated with a negative pre-exponential component; (2) the long lifetimes associated with N* and T* bands are the same and (3) the pre-exponential coefficients for the T* band emission decay are opposite in sign and of the same magnitude.[169] Analysis of the time-resolved results obtained here from PNIPAm thin films, show similar features. The short- and long- lived components of the N* and T* bands are rather close, while the pre-exponential coefficients of the T* are opposite in sign and similar in magnitude. This shows that the FE probe and its analogues may undergo reversible ESIPT reaction in the polymer. However, the additional third component for the N* emission of the probes in the polymer suggests strongly the presence of H-bonded probe-polymer complexes with different ability to undergo ESIPT. As alluded to earlier, there are two possible H-bond interactions between the FE and MFE probes that will strongly influence the ESIPT process. The interaction between the 4-carbonyl group of the probes and the amide hydrogen of the polymer has been previously reported to significantly decrease the ESIPT rates.[176] The other type of interaction (between the 3-hydroxy group of the probes and the carbonyl group of the polymer) should disrupt intramolecular H-bonding and the ESIPT process. The presence of these two H-bonding interactions could explain the additional decay time observed, which was not observed in aprotic organic solvents.[169]

For each of the probe polymer combinations, there seems to be two different fluorophore populations, the first undergoes reversible ESIPT whereas the second is strongly hydrogen bound with an inhibited ESIPT process. This is consistent with what has been previously reported for a different PNIPAm polymer and NIPAM-NtBA copolymers.[76, 88] The weak sensitivity of the lifetime data to humidity changes indicates that the observed fluorescence quenching at higher humidity is static in nature (refer to steady state data figure 39, section 3.3).
3.5 Comparison of Commercial PNIPAm versus synthesised PNIPAm

In this section we shall give a brief overview of the differences observed in data collected for commercial and synthesised PNIPAm. As mentioned in Chapter 2, for comparative purposes commercial PNIPAm will be referred to as PNIPAm1 and the synthesised PNIPAm will be referred to as PNIPAm2. We suggest that the differences noted are due to differences in molecular weight (Chapter 2). The results obtained for PNIPAm2 will be discussed in more detail in Chapter 5.

Figure 47 shows the fluorescence emission spectra collected for PNIPAm1 and PNIPAm2 films doped with FE and MFE (at 10% and 90% RH). The spectra have been normalised to allow for easier comparison of the data.

Figure 47: Normalised (to N* band) smoothed spectra of PNIPAm1 and PNIPAm2 films doped with FE and MFE collected at 10% and 90% RH.
On increasing humidity there is a red shift of the N* band accompanied by a blue shift of the T* band. The same trend was observed for films doped with FE and MFE. There appears to be two stages of wavelength shifts, a small variation at lower water content followed by a more dramatic variation at higher relative humidity’s (~50% RH). The same trend was observed for PNIPAm1 and PNIPAm2; however the magnitude of wavelength shifts differ. PNIPAm1 displays a greater N* band red shift (~ 10nm) and a smaller T* blue shift (~ 2nm) on increasing RH (from 10% to 90%) than PNIPAm2 (N* red shift of ~ 6-7 nm and T* blue shift of ~ 4-5 nm) (figure 48).

As noted previously in this chapter the large N*-T* band separation can be attributed to the significant polymer-probe hydrogen bonding and a decrease in this band separation may be attributed to a decrease of the specific polymer probe H-bonding interaction (due to water sorption). At 10% and 90% RH we observe a larger band separation in the case of PNIPAm1 than for PNIPAm2 doped with FE (indicating a greater degree of H-bond interaction for PNIPAm1 than PNIPAm2) however we observe the opposite in the case of MFE (PNIPAm2 displays a greater degree of band separation). Table 8 summarises and compares the observed differences in band positions for PNIPAm1 and PNIPAm2 films doped with FE and MFE at 10% and 90% RH.

Figure 48: Plot of variation in N* and T* band positions against increasing %RH for PNIPAm1 and PNIPAm2 films doped with (A) FE and (B) MFE at 25°C.
Plotting the log of intensity ratio (\(\log(I_{N*}/I_{T*})\)) versus relative humidity (figure 49), it can be seen that increased water content results in an increase of \(\log(I_{N*}/I_{T*})\) values. The same trend is observed for both polymers doped with FE and MFE. This increase of \(\log(I_{N*}/I_{T*})\) values with increasing humidity occurs at two different rates: first a small increase at lower humidity’s followed by a greater increase above ~50% RH. This is a result of an increase in micropolarity caused by water sorption leading to an increase in \(N^*\) emission intensity relative to \(T^*\) emission.

<table>
<thead>
<tr>
<th></th>
<th>FE 10%</th>
<th>FE 90%</th>
<th>FE 10%</th>
<th>FE 90%</th>
<th>MFE 10%</th>
<th>MFE 90%</th>
<th>MFE 10%</th>
<th>MFE 90%</th>
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<td>N*</td>
<td>489.54</td>
<td>499.93</td>
<td>564.01</td>
<td>561.93</td>
<td>487.64</td>
<td>498.47</td>
<td>567.12</td>
<td>565.07</td>
</tr>
<tr>
<td>T*</td>
<td>565.63</td>
<td>560.31</td>
<td>491.26</td>
<td>498.17</td>
<td>572.35</td>
<td>567.4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 8: Summary of the \(N^*\) red shift and \(T^*\) blue shift of PNIPAm1 and PNIPAm2 films doped with FE and MFE at 10% and 90% RH.
Figure 49: Plot of the \( \log(\text{I}_{\text{N*}}/\text{I}_{\text{T*}}) \) ratio versus increasing relative humidity at 25°C for PNIPAm1 and PNIPAm2 films doped with (A) FE and (B) MFE at 25°C. Plot of the normalised \( \text{I}_{\text{N*}}/\text{I}_{\text{T*}} \) values for PNIPAm1 and PNIPAm2 films doped with (C) FE and (D) MFE.

The \( \log(\text{I}_{\text{N*}}/\text{I}_{\text{T*}}) \) values are greater in the case of PNIPAm1 than PNIPAm2 (this is more pronounced in the case of films doped with FE). Looking at the overall change in \( \log(\text{I}_{\text{N*}}/\text{I}_{\text{T*}}) \) values between 10% and 90% RH (Table 8) it would appear that films doped with FE are displaying nearly the same overall increase in \( \log(\text{I}_{\text{N*}}/\text{I}_{\text{T*}}) \) value whereas PNIPAm2 doped with MFE displays an overall greater change. To more readily observe the changes observed they have been compared by plotting the normalised values of \( \text{I}_{\text{N*}}/\text{I}_{\text{T*}} \) ratio versus increasing humidity (figure 49). It would appear that the overall change in normalised \( \text{I}_{\text{N*}}/\text{I}_{\text{T*}} \) suggests a greater degree of water uptake by the PNIPAm1 film, suggesting that a greater molecular weight may lead to
enhanced water sorption capabilities. However this would need to be verified further.

3.6 Conclusions

In PNIPAm thin films the water adsorption process appears to follow two distinct phases. At low relative humidity the incoming water forms a primary hydration layer where the water molecules are strongly bound to the polymer and thus do not produce a very significant spectroscopic effect on the probes. The local microheterogeneity is maintained at low humidity as is evidenced by the lack of significant changes in the emission bandwidth (fwhm). However, at higher water content (relative humidity greater than 60%) the rate of change in \( \log(I_{N^*}/I_{T^*}) \), fluorescence intensity, \( N^* \) bandwidth and band shifts increase dramatically, indicating a larger change in local polarity and H-bonding of the probes with water caused by the secondary hydration of the polymer.

This combination of effects has a much larger effect on the overall ESIPT process, thus leading to the greater changes in emission parameters. The \( \log(I_{N^*}/I_{T^*}) \) value for all three fluorophores increases with MFE and BFE showing the greatest sensitivity to increasing humidity when compared with FE. However, due to BFE being strongly photobleached, it is concluded here that MFE is the best probe for assessing water uptake.

On performing emission measurements above the LCST it was clear that the PNIPAm film was still absorbing some water as similar changes in the \( \log(I_{N^*}/I_{T^*}) \) values against increasing humidity were noted, although the magnitude of these values were reduced in each case indicating that the local polarity is much less than that for measurements performed below the LCST.

Little or no change was observed in average lifetime or component lifetimes for either the \( N^* \) or \( T^* \) bands with increasing humidity. The probes present at least two populations: one undergoing reversible ESIPT, while the other is strongly bound with an inhibited ESIPT process. There was a weak sensitivity of time-resolved data to humidity changes indicating that the observed fluorescence quenching is static in

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nature. It is concluded that the water is strongly bound to the polymer and not present in a freely diffusing manner.

Differences were noted in the data collected between PNIPAm polymers of different molecular weight. Both FE and MFE appeared sensitive to the molecular weight difference and the results collected suggest that the greater the molecular weight the greater the polarity and greater water uptake ability. However we should approach these results with caution as further analysis would need to be carried out to confirm the hypothesis.

The use of the MFE dual band ratiometric fluorophore in combination with a controlled humidity chamber offers considerable promise for analysing, in detail, water uptake in thin film hydrophilic polymers. Due to the high sensitivity of the fluorescence measurement method, it is possible to observe the subtle changes that occur with small amounts of adsorbed water.

4.1 Introduction

Here we use the $E_T(30)$ method in conjunction with the controlled humidity chamber to measure the polarity of poly(NIPAM-co-EPM) thin films. Measurements were taken at various relative humidity (from 10%RH to 90%RH) to see how water uptake by the copolymer films would impact on the $E_T(30)$ measurement.

4.2 Absorption Band Position Variation

For the poly(NIPAM-co-EPM) copolymer films doped with Reichardt’s betaine dye the spectra (under all RH conditions) showed a single intramolecular charge-transfer (ICT) band. The position of this band was strongly dependant on increasing water content within the various polymer films; although the band position did not vary very much with copolymer composition with the exception of PEPm at RH < 70%. Figures 50 and 51 show the UV/Vis absorption spectra collected for the respective polymer films doped with Reichardt’s betaine dye at various humidities (10%-90% RH). The spectra have been smoothed and normalised (to the maximum of the longest wavelength absorption band) to allow for easier comparison.
Figure 50: Smoothed, normalised absorption spectra of Reichardt’s betaine dye dispersed in poly(NIPAM-co-EPM) thin films collected at different relative humidity (from 10% to 90%). Spectra were not corrected for instrument response, thus caution should be exercised when comparing these absorption spectra with other published data.
Figure 51: Smoothed, normalised absorption spectra of Reichardt’s betaine dye dispersed in poly(NIPAM-co-EPM) thin films overlaid at each relative humidity.

Figures 50 and 52 (A) show that the absorption band displays a blue or hypsochromic shift to shorter wavelengths with increasing water content in the polymer films. The blue shift in the absorption spectra indicates that the probe is located in a more polar environment i.e. with increasing water content there is an
increase in local polarity.

Figures 51 and 52 (B) show the dependence of absorption band position on copolymer composition. PEPm displays a red or bathochromic shift to longer wavelengths at RH < 70% and it is only at 90%RH that we see a somewhat systematic blue shift of the band position with increasing EPM content. Table 10 summarises the dependence of the absorption band maxima shift on both % RH and increasing fraction of EPM.

This blue shift of absorption band maxima with increasing RH has been previously observed in polymer films.[65, 74, 75] In our case the main H-bond interaction between the polymers and Reichardt’s dye is probably between the phenoxide oxygen of the dye and the polymer N-H groups. Water molecules adsorbed by the polymer have a free hydroxyl group acting as an acidic H-bond donor to the basic phenoxide oxygen (O⁻) of the dye. The increase of water content within the films leads to a change in the dye microenvironment causing a blue shift in the absorption spectra. This may be a result of an increase in electronic transition energy brought about by the stabilisation of the dipolar ground state relative to the less dipolar excited state.[65]

It is important to note that due to the hydrophilic nature of the dye it may make interpretation of the ICT band complicated. Matsuguchi et al measured the water adsorption capability of the Reichardt’s dye itself and found that it did absorb a large amount of water (80.9 mg water per gram of dye). They also concluded that aggregation of the dye may occur (especially in the case of a high content of dye) when films are exposed to water vapour and as that the dye must be dispersed on a molecular level in the polymer in order to obtain reliable data.[65] However the author also states that if the interaction is only between water molecules and the dye then the parameters measured should be identical for all polymer systems.

Looking at PNIPAm and PEPm individually with increasing RH we see a greater blue shift of the PEPm-doped film spectra on going from 10% to 90% RH (75.9 nm as compared to 48.9 nm for PNIPAm) (table 10). This suggests that PEPm is absorbing more water than PNIPAm which we would expect as we know that PEPm is more
hydrophilic in nature than PNIPAm. McGill et al report a similar finding for films of PVP and PVAc (> 100 nm shift on increasing RH) compared with PMMA (~ 35 nm shift) and PVC (15 nm shift).[74]

Figure 52: Plot of the variation in absorption band maxima against (A) increasing % RH and (B) increasing EPM content of Reichardt’s betaine dye dispersed in poly(NIPAM-co-EPM) thin films. Error bars represent the standard deviation (S.D.) from 3 replicate measurements.
<table>
<thead>
<tr>
<th>% RH</th>
<th>PNIPAm</th>
<th>90:10</th>
<th>80:20</th>
<th>70:30</th>
<th>60:40</th>
<th>PEPm</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>633.6</td>
<td>627.4</td>
<td>631.5</td>
<td>630.8</td>
<td>631.9</td>
<td>649.3</td>
</tr>
<tr>
<td>30</td>
<td>619.8</td>
<td>617.4</td>
<td>618.8</td>
<td>615.7</td>
<td>621.9</td>
<td>640.8</td>
</tr>
<tr>
<td>50</td>
<td>606.7</td>
<td>604.6</td>
<td>604.6</td>
<td>600.4</td>
<td>606.7</td>
<td>623.3</td>
</tr>
<tr>
<td>70</td>
<td>594.8</td>
<td>592.0</td>
<td>591.3</td>
<td>588.9</td>
<td>591.3</td>
<td>591.7</td>
</tr>
<tr>
<td>90</td>
<td>584.7</td>
<td>583.6</td>
<td>580.8</td>
<td>578.7</td>
<td>578.5</td>
<td>573.4</td>
</tr>
<tr>
<td>Δ Blue Shift with RH</td>
<td>48.9</td>
<td>43.8</td>
<td>50.7</td>
<td>52.1</td>
<td>53.3</td>
<td>75.9</td>
</tr>
</tbody>
</table>

Table 9: Summary of the dependence of the absorption band maxima shift (nm) on increasing EPM fraction and on increasing % RH of poly(NIPAM-coEPM) films doped with Reichardt’s betaine dye.

McGill et al refer to the phenomenon of solvent sorting [74] and due to the fact that all the polymer systems we have studied are water soluble it is worth considering the possibility of its occurrence. In a mixture of two or more solvents, solvent sorting takes place when a solute is preferentially dissolved by one solvent over another and the solute behaves as if it were dissolved only in the preferred solvent. The authors suggest that if solvent sorting were occurring in the case of polymers and water (water being the preferred solvent) then the blue shift of the absorption band maxima should be greater at low to moderate RH than at moderate to high RH (i.e. solvent sorting occurs when the dye preferentially associates with water and the observed spectral response would be of the dye interaction with water as opposed to a response from the interaction of water with the polymer). They concluded that in the case of PMMA, PVAc, and PVC, solvent sorting was not occurring because a linear variation of band position with increasing humidity was observed. However in the case of PVP (a water soluble polymer) a nonlinear variation was observed with a significant variation at low to moderate RH (shift of ~ 90 nm) but this variation was much less at higher RH. Thus the conclusion was that possibly some solvent sorting was taking place.

Figure 53 shows the individual plots (with a linear fit) of the variation of band position with RH for each of the poly(NIPAM-co-EPM) films studied. It is apparent that the wavelength shift is linear for PNIPAm and the copolymers but not for PEPm.
The non-linear response of the PEPm doped film with increasing water content suggests that there may be some solvent sorting occurring.

**Figure 53:** Plot of the variation in absorption band maxima against increasing RH of Reichardt’s betaine dye dispersed in poly(NIPAM-co-EPM) thin films. The red line in the plots shows a linear fit (Boltzman fit in the case of PEPm) which was computed via Origin.

For PNIPAm through to 60:40 films a small variation of band position with
increasing RH was noted (between 13.8 and 10.2 nm for PNIPAm and between 10 and 12.8 nm for 60:40) (table 11). However PEPm displays a greater variation at higher RH than at lower RH thus contradicting the possible occurrence of solvent sorting (where we should see a greater variation at lower RH). It is difficult to say for definite at this point whether solvent sorting is occurring, but due to the high concentration of Reichardt’s dye used (1 x 10^{-2} M) it very well may be a possibility.

<table>
<thead>
<tr>
<th>RH %</th>
<th>PNIPAm</th>
<th>90:10</th>
<th>80:20</th>
<th>70:30</th>
<th>60:40</th>
<th>PEPm</th>
</tr>
</thead>
<tbody>
<tr>
<td>10-30</td>
<td>13.8</td>
<td>10</td>
<td>12.7</td>
<td>15.2</td>
<td>10</td>
<td>8.5</td>
</tr>
<tr>
<td>30-50</td>
<td>13.2</td>
<td>12.8</td>
<td>14.2</td>
<td>15.3</td>
<td>15.2</td>
<td>17.5</td>
</tr>
<tr>
<td>50-70</td>
<td>11.8</td>
<td>12.6</td>
<td>13.3</td>
<td>11.5</td>
<td>15.3</td>
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<td>70-90</td>
<td>10.2</td>
<td>8.4</td>
<td>10.5</td>
<td>10.2</td>
<td>12.8</td>
<td>18.3</td>
</tr>
</tbody>
</table>

Table 10: Differences in absorption band blue shift on going from low to high RH.

4.3 The ET(30) Polarity Parameter

ET(30) values were calculated according to equation 1 [62]

\[
ET(30) \text{ [kcal.mol}^{-1}] = h.c.\nu_{\text{max}}.N_A = 2.859 \times 10^{-3} \cdot \nu_{\text{max}}
\]  

(1)

where \( h \) is Planck’s constant, \( c \) is the velocity of light and \( N_A \) is Avagadro’s number. For all six polymers studied ET(30) values increase with increasing RH. This indicates that the polarity of the polymers becomes greater with increased water content (figure 54 (A) and table 12). One would expect this result due to the fact that water is highly polar and thus the presence of increased amounts of water in the polymer films would enhance the polarity.
Figure 54: Dependence of the $E_T(30)$ polarity parameter on (A) increasing % RH and (B) the % of EPM content. Error bars represent the standard deviation (S.D.) from 3 replicate measurements.
<table>
<thead>
<tr>
<th>% RH</th>
<th>PNIPAm</th>
<th>90:10</th>
<th>80:20</th>
<th>70:30</th>
<th>60:40</th>
<th>PEPm</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>45.4</td>
<td>45.5</td>
<td>45.6</td>
<td>45.3</td>
<td>44.9</td>
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<tr>
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<td>±0.3</td>
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<td>45.7</td>
<td>44.5</td>
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<td>±0.2</td>
<td>±0.1</td>
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<td>70</td>
<td>49.0</td>
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<tr>
<td></td>
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<td>±0.1</td>
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</tr>
<tr>
<td>90</td>
<td>49.8</td>
<td>49.8</td>
<td>49.8</td>
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</tr>
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<td></td>
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<td>±0.1</td>
<td>±0.1</td>
<td>±0.1</td>
<td>±0.1</td>
<td>±0.4</td>
</tr>
<tr>
<td>Δ increase of $E_T(30)$</td>
<td>3.5</td>
<td>3.5</td>
<td>3.7</td>
<td>4.0</td>
<td>4.4</td>
<td>5.6</td>
</tr>
</tbody>
</table>

Table 11: Summary of the dependence of the $E_T(30)$ parameter value (kcal.mol$^{-1}$) on increasing EPM fraction of and on increasing % RH of poly(NIPAM-co-EPM) films doped with Reichardt’s betaine dye.

We don’t observe a very significant variation in $E_T(30)$ values at each RH on increasing EPM content (Figure 54 (B) and table 12). It appears that from 10% to 70% RH a high EPM content (40 and 100% EPM) leads to lower $E_T(30)$ values suggesting that increasing EPM content reduces polarity. It is only at 90% RH that we observe a small increase in $E_T(30)$ values indicating an increase in polarity with increasing EPM content.

If we look instead at the overall change in $E_T(30)$ parameter on going from 10% to 90% RH (table 12) we see an overall increase of 3.52 kcal.mol$^{-1}$ for PNIPAm increasing with increasing EPM content to 5.62 kcal.mol$^{-1}$ for PEPm. This indicates that as water is adsorbed there is a bigger change in polarity as EPM content is increased. However, this larger change in polarity (for PEPm) as water is incorporated is probably due to solvent sorting of the dye into the aqueous phase.

4.4 Conclusions

The polarity of poly(NIPAM-co-EPM) films were evaluated by means of the $E_T(30)$ solvatochromic polarity parameter. From the results obtained it is clear that
increasing water content in the copolymer films results in increased polarity (as would be expected due to the highly polar nature of water). Absorption band maxima positions and $E_{(T)}(30)$ values did not vary much with copolymer composition (with the exception of PEPm which displayed a red shift in band maxima and lower $E_{(T)}(30)$ values at RH < 70%, suggesting a decrease in polarity). Due to the non-linear correlation of band position vs RH for PEPm it is suggested here that solvent sorting of the dye into the aqueous phase is occurring.

5.1 Introduction

Poly(ethylpyrrolidone-methacrylate) (PEPM) is a new thermo-responsive polymer alternative and was developed with a view to biomaterial applications due to the excellent biocompatibility of the pyrrolidone ring (as has been shown previously in polymers such as poly(vinyl-pyrrolidone).[183] It is a synthetic, amphiphilic, linear polymer which has a LCST at ~ 60ºC. By copolymerisation of NIPAM with EPM it was hoped that resulting copolymers would yield copolymers that would retain PNIPAm's sharp phase transition, have a LCST close to physiological temperature and retain EPM's biocompatible nature. Increasing the fraction of EPM results in increased hydrophilicity and thus an increase in LCST. Here we study water uptake in thin NIPAM/EPM films supported on quartz substrates by studying the fluorescence emission properties of FE and MFE. These two probes were chosen as they displayed the greatest sensitivity to water uptake in thin PNIPAm films (Chapter 3).

5.2 Equilibration

As noted in Chapter 3 it was critical to first establish equilibration profiles for each of the NIPAM-EPM copolymers to determine the amount of time required at each RH step to achieve equilibration between the chamber environment and the polymer film. Thus, initial measurements were conducted every ten minutes at 25ºC until equilibration was achieved at each humidity level (figure 55 and figure 56).

Equilibration time is not only a function of water content level but also of polymer composition. As can be seen from figures 55 and 56, equilibration time appears to decrease with increasing EPM content. As we have seen from LCST

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*PNIPAm coatings tend to be poorly biocompatible/cell compatible. Copolymerisation of PNIPAm with more biocompatible monomers (such as EPM) may overcome this lack of compatibility. Films of poly(NIPAM-co-EPM) hosted cells successfully to monolayer. Upon temperature reduction cells detached from the copolymers while maintaining cell to cell junctions. These results indicated that the copolymers were highly cell compatible and are potentially useful for a range of biomedical applications. These results were found by Dr. Maria Nash. Paper yet to be published.[178]
measurements (Chapter 2), increasing EPM content increases the hydrophilicity of the copolymers, thus one could deduce from the equilibration profiles presented that the increasing hydrophilicity of the copolymer films leads to quicker equilibration. Incorporation of the EPM monomer decreases the transition enthalpy with a modest increase of transition temperature. This is evidenced by the small variation of LCST with low amounts of EPM in NIPAm-EPM copolymers (Chapter 2). Such behaviour may indicate that EPM rich copolymers retain more water in the collapsed state and this is corroborated by the lower contact angles obtained for NIPAm-EPM copolymers in the collapsed state (Chapter 2). Also from LCST and contact angle measurements we know that PEPm is more hydrophilic than PNIPAm, so this coupled with the smaller enthalpy of transition for EPM rich copolymers is possibly why we see enhanced equilibration with increasing EPM content. The log of intensity ratio (log(I_{N^*}/I_{T^*})) values were used as these values provide the best indication of changes in the local environment due to water sorption.
Figure 55: Equilibration profiles for NIPAM/EPM copolymer films doped with FE. Measurements taken at 25°C with a ten minute interval.
<table>
<thead>
<tr>
<th></th>
<th>10% RH</th>
<th>20% RH</th>
<th>30% RH</th>
<th>40% RH</th>
<th>50% RH</th>
<th>60% RH</th>
<th>70% RH</th>
<th>80% RH</th>
<th>90% RH</th>
</tr>
</thead>
<tbody>
<tr>
<td>PNIPAm</td>
<td>0.049</td>
<td>0.058</td>
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<td>0.060</td>
<td>0.065</td>
<td>0.083</td>
<td>0.106</td>
<td>0.125</td>
<td>0.141</td>
</tr>
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<tr>
<td></td>
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<td>0.049</td>
<td>0.056</td>
<td>0.076</td>
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<tr>
<td></td>
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<td>0.0011</td>
<td>0.0011</td>
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<td>0.0047</td>
<td>0.0051</td>
<td>0.0042</td>
<td>0.0008</td>
</tr>
<tr>
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<td>-0.021</td>
<td>-0.011</td>
<td>-0.002</td>
<td>0.01</td>
<td>0.034</td>
<td>0.069</td>
<td>0.104</td>
<td>0.127</td>
</tr>
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</tr>
<tr>
<td></td>
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<td>0.0005</td>
<td>0.0016</td>
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<td>0.0042</td>
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<td>0.0025</td>
<td>0.0022</td>
</tr>
<tr>
<td>70:30</td>
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</tr>
<tr>
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<td>0.0009</td>
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<td>0.0047</td>
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<td>60:40</td>
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</tr>
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<td>0.0006</td>
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<td>0.0015</td>
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<td>-0.356</td>
<td>-0.302</td>
<td>-0.236</td>
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<td>0.0004</td>
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</table>

Table 12: Table showing the mean and standard deviation of $\log(I_{N^+}/I_{T^+})$ equilibration values at each level of RH for polymer films doped with FE.
Figure 56: Equilibration profiles for NIPAM/EPM copolymer films doped with MFE. Measurements taken at 25°C with a 10 minute interval.
### Table 13: Table showing the mean and standard deviation of \( \log(I_{N^*}/I_{T^*}) \) equilibration values at each level of RH for polymer films doped with MFE.

<table>
<thead>
<tr>
<th></th>
<th>10%</th>
<th>20%</th>
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<th>40%</th>
<th>50%</th>
<th>60%</th>
<th>70%</th>
<th>80%</th>
<th>90%</th>
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</tr>
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<tr>
<td>PEPm</td>
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<td>-0.553</td>
<td>-0.515</td>
<td>-0.466</td>
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<td>-0.325</td>
<td>-0.238</td>
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<td>0.0005</td>
<td>0.0008</td>
<td>0.0027</td>
<td>0.0135</td>
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</tr>
</tbody>
</table>

### 5.3 Fluorescence Spectroscopy.

The fluorescence emission spectra for poly(NIPAM-co-EPM) films doped with FE and MFE are were collected at 25°C and display two well resolved emission bands (which can be assigned to the emission of the normal (N*) and tautomeric (T*) excited states) [109, 153]. On increasing relative humidity, water is adsorbed by the respective copolymer films resulting in a wavelength shift of both the N* and T* emission bands. Not only do the band positions vary with increasing relative humidity but we also note a variation of band position and width with copolymer composition.

To facilitate easier comparison and discussion of all the data collected in this body of work we will first discuss the 100% PNIPAm2 case along with the 100% PEPm case and compare the data collected for both polymers. This will be done in two stages: (1) description of observations made in the case of films doped with FE (2) description of observation in the MFE case. Finally we shall discuss the results.
collected as EPM content is increased in the copolymer films doped with both FE and MFE.

5.3.1 Comparison of PNIPAm2 with PEPm films doped with FE and MFE.

PEPm as we know from LCST and contact angle measurements (Chapter 2) is more hydrophilic in nature than PNIPAm2. This is observed here by the dramatic differences observed in emission properties of FE and MFE probes as humidity is increased from 10% to 90% RH. As water is adsorbed by the polymer films (below the LCST) we observe changes in emission intensity, there is a wavelength shift of both emission bands and the emission intensity ratio changes. Figure 57 shows the normalised emission spectra collected for PNIPAm and PEPm films doped with FE and MFE. As humidity increases there is a red shift of the N* band accompanied by a blue shift of the T* band. This same trend was observed for FE and MFE.
Figure 57: Normalised (to N* band) smoothed spectra of PNIPAm and PEPm films doped with FE and MFE collected at different relative humidity (between 10% and 90%).

5.3.1.1 PEPm Films doped with FE.

In the case of PEPm films the magnitude of the observed wavelength shifts with increased water sorption is dramatically increased compared to PNIPAm2 e.g. there is a 28 nm red shift of the N* band accompanied by a T* blue shift of 13 nm. We observe two stages of wavelength shifts, a small variation at lower water content followed by a larger variation at higher relative humidity’s (above ~50% RH). This same trend is observed for both PNIPAm2 and PEPm films (figure 58).
Figure 58: Plot of variation in $N^*$ and $T^*$ band positions against increasing %RH for PNIPAm2 and PEPm films doped with FE.\(^5\)

Not only to we see a variation of band position with increasing humidity, we also observe a difference in band position with the differences in chemical composition between PNIPAm2 and PEPm. For instance when overlaying the spectra collected for PNIPAm2 and PEPm at 10% RH we can see a 2 nm red shift of the $N^*$ band accompanied by an 11 nm red shift of the $T^*$ band. At 90% RH the situation is very different. We observe a 24 nm red shift of the $N^*$ band and a 2 nm red shift of the $T^*$ band (figure 59 and table 14).

Figure 59: Normalised (to $N^*$ band) smoothed, overlayed spectra of PNIPAm and PEPm films doped with FE collected at 10% and 90% RH.

\(^5\) PEPm was not measured above its LCST as a temperature of > 60°C was outside the capabilities of the humidity chamber (max. temperature capability of the chamber ~ 39°C).
As we have mentioned the large N*-T* band separation observed for the 3-HF doped polymer films can be attributed to the significant polymer-probe hydrogen bonding. At 10% RH PNIPAm2 films display an N*-T* band separation of ~72 nm whereas PEPm films display an N*-T* separation of 82 nm. The larger separation observed for PEPm films may suggest a greater degree of polymer-probe hydrogen bonding at this low relative humidity. At 90% RH the situation is reversed. We see a greater degree of band separation for PNIPAm2 (~ 61 nm) compared to PEPm (~ 40 nm). Due to increased hydrophilicity of the PEPm polymer there is possibly a greater interruption of polymer-probe H-bonding with increased water content and it is suggested here that at 90% RH the probe is experiencing a more solvent like environment than under the same conditions for PNIPAm2. This is consistent with the observation of solvent sorting for PEPm films doped with Reichardt's dye (Chapter 4).

There are also significant changes observed in the intensity of the N* and T* bands (figure 60). Up to ~50% RH, a gradual increase in N* intensity is observed followed by a much steeper increase (this is consistent for both PNIPAm2 and PEPm films). In contrast, the T* intensity is very different. PNIPAm2 films display a slight increase up to ~ 60% RH followed by a slight decrease, whereas PEPm films show an overall decrease in T* intensity across all humidity levels (figure 60). It should be noted that for both PNIPAm2 and PEPm the change of N* intensity with increasing RH appears to be more pronounced than that of T* intensity. PEPm films display a more dramatic variation when compared to PNIPAm2 in that N* intensity increases by ~100% and T* intensity decreases by ~33%.

<table>
<thead>
<tr>
<th></th>
<th>FE 10% N*</th>
<th>FE 90% N*</th>
<th>FE 10% T*</th>
<th>FE 90% T*</th>
</tr>
</thead>
<tbody>
<tr>
<td>PNIPAm2</td>
<td>492.71</td>
<td>498.9</td>
<td>565.63</td>
<td>560.31</td>
</tr>
<tr>
<td>PEPm</td>
<td>494.9</td>
<td>522.77</td>
<td>576.58</td>
<td>562.8</td>
</tr>
<tr>
<td>Total Shift</td>
<td>~2 nm</td>
<td>~24 nm</td>
<td>~11 nm</td>
<td>~2 nm</td>
</tr>
</tbody>
</table>

Table 14: Summary of the N* and T* red shift between PNIPAm2 and PEPm films doped with FE at 10% and 90% RH (at 25ºC).
Figure 60: Variation of N* and T* intensity with increasing humidity of PNIPAm2 and PEPm films doped with FE.

Plotting the integral intensity versus increasing humidity (figure 61) it is clear that on increasing RH the overall fluorescence intensity increases for PNIPAm2 and PEPm films (the response appears to be greater in the case of PNIPAm2 films). This may seem contradictory in light of the results shown above for the variations in relative intensity of the FE doped PEPm film (due to the decrease in T* band intensity). However as mentioned the N* band intensity increase is larger than the T* intensity decrease thus this may be the reason that we observe an overall increase in the integral intensity with increased water sorption.
Figure 61: Plot of normalised integral intensity$^6$ versus relative humidity for PNIPAm2 and PEPm films doped with FE.

There are significant differences in the full width at half maximum for PNIPAm2 and PEPm films doped with FE. We observe a decrease in N* bandwidth for both polymers (figure 62) indicating that with increased water content FE is experiencing a narrower range of microenvironments. Aside from the decrease with increasing humidity we also notice a decrease of values obtained for PEPm in comparison to PNIPAm2, indicating FE senses a narrower range of microenvironments in the PEPm films than the PNIPAm2 film. In contrast to this an increase in T* bandwidth is observed (figure 62), indicating a greater degree of heterogeneity (this increase being more pronounced for PEPm than PNIPAm).

$^6$ The area under the curve from 425nm-700nm: calculated in Origin ver. 7.0 by means of the calculus-integrate function.
Figure 62: Plot of N* and T* bandwidths (full widths at half maximum, nm) for PNIPAm2 and PEPm films doped with FE versus increasing humidity.

Plotting the log of intensity ratio ($\log(I_{N^*}/I_{T^*})$) versus increasing humidity (figure 63 (A)), it can be seen that increased water content results in an increase of $\log(I_{N^*}/I_{T^*})$ values. For PNIPAm2 films the increase of $\log(I_{N^*}/I_{T^*})$ values occurs at two different rates (as was observed for commercial PNIPAm in Chapter 3). First there is a small increase at lower humidity’s followed by a greater increase above ~50% RH. In contrast PEPm films display a more exponential dependence of $\log(I_{N^*}/I_{T^*})$ values on increasing RH indicating a more uniform hydration process. This increase in log ratio values occurs due to a dramatic increase in micropolarity caused by water sorption leading to an increase in N* emission relative to T* emission.[109] This is in agreement with observations made in Chapter 4 where $E_T(30)$ values increased with increasing RH for PNIPAm2 and PEPm films doped with Reichardt’s dye, indicating an increase in polarity as water is adsorbed by the polymer.

Plotting the normalised\(^7\) values of $I_{N^*}/I_{T^*}$ ratio versus increasing humidity it is clear to see that PEPm films display larger changes in the ratio values with increased water content than PNIPAm2 films. Increasing humidity from 10% to 90% PNIPAm2 displays an overall change of ~ 0.281 in $I_{N^*}/I_{T^*}$ whereas PEPm displays an overall change of ~ 0.807 suggesting that (1) the PEPm film adsorbs water at an increased

\(^7\) $I_{N^*}/I_{T^*}$ ratio values were normalised to allow for easier comparison of PNIPAm and PEPm data.
rate and (2) that the FE probe senses the increased water uptake ability of the PEPm polymer film. Table 15 summarises and compares the differences in intensity ratio values for PNIPAm2 and PEPm film at each 10% rise in humidity. Overall at each rise in humidity level PEPm displays a greater change in ratio value compared to PNIPAm2. Plotting these differences in ratio values against the differences in RH (figure 64) further indicates the presence of a more uniform hydration process in the case of PEPm.

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**Figure 63:** Plot of (A) log$(I_{N^*}/I_{T^*})$ ratio and (B) $(I_{N^*}/I_{T^*})$ ratio normalized to the value recorded at 90% RH versus increasing RH for PNIPAm2 and PEPm films doped with FE.

<table>
<thead>
<tr>
<th>RH (%) Increase</th>
<th>PNIPAm2 $I_{N^<em>}/I_{T^</em>}$ Ratio Change</th>
<th>PEPm $I_{N^<em>}/I_{T^</em>}$ Ratio Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>10-20</td>
<td>0.024</td>
<td>0.032</td>
</tr>
<tr>
<td>20-30</td>
<td>0.027</td>
<td>0.041</td>
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<tr>
<td>30-40</td>
<td>0.012</td>
<td>0.055</td>
</tr>
<tr>
<td>40-50</td>
<td>0.022</td>
<td>0.079</td>
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<tr>
<td>50-60</td>
<td>0.039</td>
<td>0.11</td>
</tr>
<tr>
<td>60-70</td>
<td>0.08</td>
<td>0.143</td>
</tr>
<tr>
<td>70-80</td>
<td>0.047</td>
<td>0.174</td>
</tr>
<tr>
<td>80-90</td>
<td>0.054</td>
<td>0.173</td>
</tr>
</tbody>
</table>

**Table 15:** Summary of the differences in intensity ratio values induced by a change in relative humidity for PNIPAm2 and PEPm films doped with FE.
Figure 64: Plot of the difference in intensity value induced by a change in relative humidity for PNIPAm and PEPm films doped with FE.

5.3.1.2 Polymer Films doped with MFE.

As water is adsorbed by PNIPAm films doped with MFE we observe a 7 nm red shift of the N* band accompanied by a 4 nm blue shift of the T* band. In contrast PEPm displays an increased N* band red shift of 13 nm on increasing RH (from 10% to 90%), however the T* band displays the same 4 nm blue shift as observed in the case of PNIPAm. There are two stages of wavelength shifts observed (consistent for both polymers): a larger variation at higher water content (above ~50% RH) preceded by a small variation at lower relative humidity’s (figure 65).
When overlaying the spectra collected at 10% and 90% RH for PNIPAm2 and PEPm films doped with MFE, we see similar changes to those observed for FE. Comparing the spectra collected for PNIPAm2 with that of PEPm at 10% RH, there is a red shift of the N* band of ~ 4 nm accompanied by a T* red shift of ~7 nm. At 90% RH there are dramatic differences; the N* and T* bands are now red shifted by ~ 15 nm and 9 nm respectively (figure 66 and table 16).

Figure 65: Plot of variation in N* and T* band positions against increasing %RH for PNIPAm2 and PEPm films doped with MFE.

Figure 66: Normalised (to N* band) smoothed, overlayed spectra of PNIPAm2 and PEPm films doped with MFE collected at 10% and 90% RH.

---

8 PEPm was not measured above its LCST as a temperature of > 60°C was outside the capabilities of the humidity chamber (max. temperature capability of the chamber ~ 39°C).
Table 16: Summary of the N* and T* red shift between PNIPAm2 and PEPm films doped with MFE at 10% and 90% RH (at 25°C).

<table>
<thead>
<tr>
<th></th>
<th>MFE 10% N*</th>
<th>MFE 90% N*</th>
<th>MFE 10% T*</th>
<th>MFE 90% T*</th>
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<td>PNIPAm2</td>
<td>491.26</td>
<td>498.17</td>
<td>572.35</td>
<td>567.4</td>
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<tr>
<td>PEPm</td>
<td>495.99</td>
<td>513.75</td>
<td>579.75</td>
<td>576.58</td>
</tr>
<tr>
<td>Total Shift</td>
<td>~4nm</td>
<td>~15nm</td>
<td>~7nm</td>
<td>~9nm</td>
</tr>
</tbody>
</table>

Due to the significant polymer-probe H-bonding interaction a large N*-T* band separation is observed for PEPm films at 10% and 90% RH, this is ~83 nm (N*) and ~63 nm (T*). The smaller degree of band separation in PEPm at 90%RH (compared to PNIPAm2) suggests a greater interruption of the H-bonding interaction which may be due to a greater water uptake ability of the PEPm due to its more hydrophilic character.

There are also variations observed in the relative intensity of both the emission bands with increasing humidity (figure 67). For PEPm, we see a more regular exponential change in N* and T* intensity as the RH increases. It is almost identical to the FE case and is very different compared to PNIPAm2. An overall decrease in T* band intensity is observed for PEPm films on going form 10% to 90% RH however in contrast to this PNIPAm films first display an increase in T* intensity up to ~60% RH followed by a decrease at higher relative humidity’s.

Figure 67: Variation of N* and T* intensity with increasing humidity of PNIPAm2 and PEPm films doped with MFE.
As humidity increases, overall fluorescence intensity increases as can be seen from the response of integral intensity of the spectra collected (figure 68). Again observed is the appearance of the two stage behaviour with increasing water content. The same trend was observed for both PNIPAm2 and PEPm films, however the magnitude of the PNIPAm2 integral intensity is greater than that observed for PEPm. The smaller increase in intensity observed for the PEPm doped film may seem surprising due to the consistent decrease in T* band intensity on increasing RH reported above. However, taking a closer look at the T* intensity decrease and N* intensity increase it is clear that the overall N* band intensity increase (over ~ 100%) is larger than the overall T* band intensity decrease (~ 32%). This may account for the observed increase in integral intensity of the spectra collected.

![Figure 68](image.png)

**Figure 68**: Plot of normalised integral intensity versus relative humidity for PNIPAm and PEPm films doped with MFE.

We observe similar changes in the full width at half maximum for PNIPAm2 and PEPm films doped with MFE as was noted in the case of FE doped films. There is a decrease in N* bandwidth for both polymers (figure 69) (indicating that MFE is
experiencing a narrower range of microenvironments both with increased water content and on changing chemical composition) accompanied by an increase in T* bandwidth (again the increase being more pronounced for PEPm doped films) (figure 69), indicating a greater degree of heterogeneity.

![Graph showing N* and T* bandwidths (full widths at half maximum, nm) for PNIPAm2 and PEPm films doped with MFE versus increasing humidity.](image)

**Figure 69:** Plot of N* and T* bandwidths (full widths at half maximum, nm) for PNIPAm2 and PEPm films doped with MFE versus increasing humidity.

As humidity increases there is an increase in \( \log(I_{N^*}/I_{T^*}) \) values occurring in two different stages: a small increase up to ~ 50%RH followed by a greater increase at higher relative humidity’s (figure 69 (A)). This two stage behaviour is noted only in the case of PNIPAm2 doped films. \( \log(I_{N^*}/I_{T^*}) \) values appear to increase in a more linear fashion upon increased water content within PEPm films. The observed increase in \( \log(I_{N^*}/I_{T^*}) \) values may be attributed to an increase in the local micropolarity induced by water sorption leading to an increase in N* emission.

Figure 69 (B) shows the changes in normalised \( I_{N^*}/I_{T^*} \) values as humidity is increased. It may be deduced from the plot that PEPm displays a greater rate of change in \( I_{N^*}/I_{T^*} \) ratio values in response to water uptake in comparison to PNIPAm2. On going from 10% to 90%RH PEPm displays a total \( I_{N^*}/I_{T^*} \) difference of 0.621 whereas PNIPAm2 values show a difference of 0.219. Table 17 summarises and compares the differences in intensity ratio values for PNIPAm2 and PEPm films at each 10% rise in humidity. Plotting these differences in ratio values against the differences in RH (figure 70) indicates that at each rise in humidity level PEPm
displays a greater change in ratio value compared to PNIPAm.

Figure 70: Plot of (A) log($I_{N^*}/I_{T^*}$) ratio and (B) ($I_{N^*}/I_{T^*}$) ratio normalized to the value recorded at 90% RH versus increasing RH for PNIPAm2 and PEPm films doped with MFE.

<table>
<thead>
<tr>
<th>RH (%) Increase</th>
<th>PNIPAm2 $I_{N^<em>}/I_{T^</em>}$ Ratio Change</th>
<th>PEPm $I_{N^<em>}/I_{T^</em>}$ Ratio Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>10-20</td>
<td>0.016</td>
<td>0.017</td>
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<td>20-30</td>
<td>0.009</td>
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<td>80-90</td>
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</table>

Table 17: Summary of the differences in intensity ratio values induced by a change in relative humidity for PNIPAm2 and PEPm films doped with MFE.
Figure 71: Plot of the difference in intensity value induced by a change in relative humidity for PNIPAm2 and PEPm films doped with MFE.

5.3.2 Effect of increasing EPM content in copolymer films.

The spectra collected for poly(NIPAM-co-EPM) colpolymer films doped with FE (figure 72) and MFE (figure 73) are shown below. As was observed in the case of PNIPAm2 and PEPm doped films we see a red shift of the N* band and a blue shift of the T* band on increasing RH for all copolymer types (figures 74 and 75).
Figure 72: Normalised (to N* band) smoothed spectra of NIPAM/EPM copolymer films doped with FE collected at different relative humidity (between 10% and 90%).
Figure 73: Normalised (to N* band) smoothed spectra of NIPAM/EPM copolymer films doped with MFE collected at different relative humidity (between 10% and 90%).
Figure 74: Plot of variation in N* and T* band positions against increasing %RH for NIPAM/EPM films doped with FE.
Figure 75: Plot of variation in N* and T* band positions against increasing %RH for NIPAM/EPM films doped with MFE.

Both FE and MFE are very sensitive to the subtle differences in the chemical composition of the copolymer films. With increasing EPM content we observe a red shift of both the N* and T* emission bands, this shift becoming greater with increased water content (figure 76). The N* and T* band positions for copolymer films doped with FE and MFE are summarised in Table 18. The N*-T* band separation increases with increasing EPM content at 10% RH (indicating an increase in copolymer-probe H-bonding interaction as EPM content is increased), but we see a decrease in band separation with increased EPM at 90% RH. The smaller degree of band separation at higher RH indicates a greater interruption of this H-bonding interaction which may be due to the increasing hydrophilicity of the copolymer films as EPM content is
increased.

Figure 76: Position of \(N^*\) and \(T^*\) band maxima as a function of EPM content of NIPAM/EPM copolymer films doped with: (A) & (B) FE and (C) & (D) MFE, across all humidity levels.

<table>
<thead>
<tr>
<th></th>
<th>FE 10%</th>
<th>FE 90%</th>
<th>FE 10%</th>
<th>FE 90%</th>
<th>MFE 10%</th>
<th>MFE 90%</th>
<th>MFE 10%</th>
<th>MFE 90%</th>
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<tbody>
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<td>% EPM</td>
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<td>T*</td>
<td>(N^*)</td>
<td>T*</td>
<td>(N^*)</td>
<td>T*</td>
<td>(N^*)</td>
<td>T*</td>
</tr>
<tr>
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<td>500.35</td>
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<td>497.81</td>
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<td>569.52</td>
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<td>498.17</td>
<td>574.46</td>
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<td>70:30</td>
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<td>570.58</td>
<td>563.15</td>
<td>490.17</td>
<td>499.62</td>
<td>575.87</td>
<td>571.99</td>
</tr>
<tr>
<td>60:40</td>
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<td>571.99</td>
<td>564.92</td>
<td>491.99</td>
<td>500.71</td>
<td>576.58</td>
<td>573.05</td>
</tr>
</tbody>
</table>

Table 18: Summary of the \(N^*\) and \(T^*\) red shift on increasing EPM fraction of poly(NIPAM-co-EPM) films doped with FE and MFE at 10% RH and 90% RH.
As EPM content is increased we see dramatic variations in the intensity of both the N* and T* bands. The intensity of both emission bands is strongly dependant on copolymer composition (figures 77 and 78).

Figure 77: Normalised fluorescence spectra (to the maximum of both the N* and T* bands) of NIPAM/EPM copolymer films doped with FE.
Figure 78: Normalised fluorescence spectra (to the maximum of both the N* and T* bands) of NIPAM/EPM copolymer films doped with MFE.

The spectra shown above have been normalised to allow for easier comparison of the data presented. It is very clear that increasing the EPM content in the copolymer films leads to an increase in T* band intensity accompanied by a decrease in N* band intensity. These same trends are observed for films doped with both FE and MFE.

As humidity is increased we again see variations of N* and T* intensities for all copolymer films. In the case of films doped with FE (figure 79) we see a gradual increase of N* band intensity up to ~ 50%RH followed by a much steeper increase at higher relative humidity’s. The T* intensity behaves very differently. For 90:10 films we initially observe a slight increase up to ~ 60% RH followed by a decrease at higher water content. However for 80:20, 70:30 and 60:40 doped films we observe an overall decrease across all humidity levels. In all cases the overall N* intensity increase is larger than the overall T* intensity decrease.

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9 Normalising to the N* band maximum one can observe the changes of the T* band; likewise normalising to the maximum of the T* band one can observe the variations of the N* band.
Copolymer films, with low EPM content (90:10 and 80:20) doped with MFE display the same trend in N* and T* variation on increasing RH as was noted for FE doped films. However for 70:30 and 60:40 doped films we see a more regular exponential change in N* and T* intensity as water content is increased (similar as to what was observed in the case of PEPm films doped with MFE) (figure 80). As noted for FE doped films the N* intensity increase is greater than the T* intensity decrease with the exception of 70:30 and 60:40 MFE doped films where the T* intensity decrease outweighs the N* intensity increase.

Figure 79: Variation of N* and T* intensity with increasing humidity of copolymer films doped with FE.
Figure 80: Variation of $N^*$ and $T^*$ intensity with increasing humidity of copolymer films doped with MFE.

The changes observed in FWHM with increasing humidity for the different copolymer films doped with FE and MFE display the same trends as previously outlined for PNIPAm2 and PEPm films. We see a decrease in $N^*$ bandwidth accompanied by an increase in $T^*$ bandwidth (figures 81 and 82).
Figure 81: Plot of N* bandwidths (full widths at half maximum, nm) for all four copolymers doped with FE and MFE versus increasing humidity.
Figure 82: Plot of T* bandwidths for all four copolymers doped with FE and MFE versus increasing humidity.

Plotting the normalised integral intensity versus increasing humidity for copolymer films doped with FE we see an overall two-stage increase in fluorescence intensity as humidity is increased (as was observed for PNIPAm2 and PEPm films). Even though we saw a decrease in T* intensity with increasing RH (figure 79) as mentioned above the increase in N* intensity is greater than the decrease in T* intensity thus leading to the increase in integral intensity.

In the case of MFE, we observe an overall increase for 90:10 and 80:20 doped films but in contrast we see a decrease in overall fluorescence intensity for 70:30 and 60:40 doped films. Again this relates back to the magnitude of N* and T* relative intensity changes previously outlined for these copolymer films. For 90:10 and 80:20 doped films the increase in N* band intensity is greater than the T* intensity decrease but the situation is reversed for 70:30 and 60:40 doped films thus resulting in the
decrease in integral intensity with increased RH.

Figure 83: Plot of normalised integral intensity versus relative humidity for thin poly(NIPAM-co-EPM) films doped with FE and MFE.

Plotting the log of intensity ratio \( \log(I_{N*/I_{T*}}) \) versus increasing humidity for all copolymers doped with FE and MFE (figure 81), we see an increase in \( \log(I_{N*/I_{T*}}) \) values with increasing water content. As observed previously (for PNIPAm and PEPm films) we see the increase of \( \log(I_{N*/I_{T*}}) \) occurring at two different rates: first a small increase at lower RH followed by a greater increase above \( \sim 50\% \) RH. This occurs due to the dramatic increase in micropolarity caused by water sorption leading to an increase in N* emission relative to T* emission (this is in line with results obtained in Chapter 4 where we saw an increase in polarity for all copolymers as water content increased). As the EPM content is increased, the plot of \( \log(I_{N*/I_{T*}}) \) versus increasing RH becomes more linear indicating a more uniform hydration process. This may be
explained by the increased hydrophilicity of the copolymers as EPM content is increased (section 5.2).

Figure 84: Dependence of log($I_{N^*}/I_{T^*}$) ratio on increasing humidity for poly(NIPAM-co-EPM) films doped with FE and MFE.

Plotting the normalised values of the $I_{N^*}/I_{T^*}$ ratios versus increasing humidity (figure 82) shows that both FE and MFE seem to be equally sensitive in monitoring water uptake in all copolymer systems analysed. However, the slightly larger changes in the ratio values as a function of increasing humidity recorded for MFE make it a marginally better probe for studying water uptake dynamics in thin polymer films.
Above their respective LCST’s these copolymers exist in a more hydrophobic state and therefore the rate of water uptake should be decreased. Measurements performed above the LCST (at 38°C) clearly show an increase in \( \log(I_{N^*/I_{T^*}}) \) with increasing RH (figures 83 and 84). The \( \log(I_{N^*/I_{T^*}}) \) are in all cases very slightly lower than those obtained from measurements performed at 25°C; this indicates the less polar environment for the condensed polymer state above the LCST. Also the lower \( \log(I_{N^*/I_{T^*}}) \) values indicate that the copolymers display less of an affinity for water above the LCST.

Figure 85: Plot of the \( (I_{N^*/I_{T^*}}) \) ratio normalized to the value recorded at 90% RH.
Figure 86: Plot of $\log(I_N/I_T^*)$ ratios against increasing %RH for NIPAM/EPM films doped with FE above and below the LCST.
Figure 87: Plot of log(I_N*/I_T*) ratios against increasing %RH for NIPAM/EPM films doped with MFE above and below the LCST.

The dependence of log(I_N*/I_T*) ratio on the copolymer composition (figure 85) clearly indicates that increasing the EPM content leads to a reduction in log(I_N*/I_T*) values. The same observation was made for both FE and MFE. One may contend that this decrease in log(I_N*/I_T*) values is also an indication of less water uptake as EPM content is increased (due to the lower log(I_N*/I_T*) values obtained when measurements were performed above the LCST). However, this is not the case, as we know from LCST and contact angle measurements (Chapter 2) that increasing the EPM content increases the hydrophilicity thus suggesting an increase in hydration process. We see an overall increase in log(I_N*/I_T*) values as EPM content is increased (on going from 10% to 90% RH) (figure 86) therefore suggesting an increase in hydrophilicity and water uptake as EPM content is increased.
Figure 88: $\log(I_{N^*/I_{T^*}})$ as a function of EPM content of NIPAM/EPM copolymer films doped with: (A) FE and (B) MFE, across all humidity levels.

Figure 89: Plot of the $\log(I_{N^*/I_{T^*}})$ ratio versus increasing relative humidity at 25°C for poly(NIPAM-co-EPM) films doped with (A) FE and (B) MFE.
6 Conclusions

We have shown that using a controlled humidity chamber one can easily study water uptake in thin thermoresponsive polymer films using spectroscopic methods. For the fluorescence measurements, dual band ratiometric 3HF probes were chosen as their emission properties are sensitive to local polarity and H-bonding effects. Also one may gain a more comprehensive picture of how water uptake affects the polymer microenvironment due to the availability of multiple parameters associated with these probes.

Fluorescence analysis of PNIPAm1 films showed that hydration occurs in two distinct phases and at higher relative humidity we observed a dramatic increase in the rate of change of \( \log(I_{N^*}/I_{T^*}) \), fluorescence intensity, \( N^* \) bandwidth, and band shifts indicating a larger change in local polarity and H-bonding of the probes with water. Measurements above and below the LCST displayed similar results but with different magnitudes. The lower log\((I_{N^*}/I_{T^*})\) values obtained above the LCST indicated a less polar environment. MFE and BFE showed the greatest sensitivity to increasing humidity, however due to BFE being photobleached we conclude that MFE is the best probe for studying water uptake. Little change in lifetimes for either the \( N^* \) or \( T^* \) band with increasing humidity indicated that observed fluorescence quenching was static in nature. It is concluded that the water is strongly bound to the polymer and not present in a freely diffusing manner.

FE and MFE were not only sensitive to water sorption but were also sensitive to differences in molecular weight. PNIPAm1 (MW 20,000-25,000) and PNIPAm2 (MW 10,000) displayed similar trends in emission properties with increasing RH i.e. both displayed a two phase hydration process. However, the data indicated that PNIPAm1 was adsorbing more water than PNIPAm2 as the log\((I_{N^*}/I_{T^*})\) were of a greater magnitude and displayed a greater overall change than for PNIPAm1. Furthermore the lower log\((I_{N^*}/I_{T^*})\) values obtained for PNIPAm2 suggest a less polar environment.

Using the \( E_T(30) \) solvatochromic parameter to evaluate the polarity of poly(NIPAM-co-EPM) films, it was clear that increasing RH resulted in increased
polarity. With increasing humidity we observed a dramatic blue shift of absorption band maxima for both PNIPAm2 and PEPm, indicating an increase in polarity as water is adsorbed. However, when plotting these results we saw a linear correlation of band shift versus RH for PNIPAm2 whereas PEPm displayed a sigmoidal correlation. This non-linear response of PEPm indicates that solvent sorting may be occurring. Also at RH up to 70% PEPm displayed an absorption band red shift and lower $E_T(30)$ values when compared to PNIPAm2 indicating a less polar environment.

As EPM content was increased in the copolymer films the same variation in band position and $E_T(30)$ values was noted with increasing RH as was noted for PNIPAm2 and PEPm (indicating increased water content increases the polarity). All copolymer films displayed a linear correlation of band position with RH. When correlating band position and $E_T(30)$ values with EPM content little or no variation was noted indicating that increasing EPM content in the films has no impact on polarity.

To fully understand and interpret the results obtained from the fluorescence analysis of the poly(NIPAM-co-EPM) films we need to look at the results separately in terms of water uptake and copolymer composition because the probes were not only sensitive to water uptake but were extremely sensitive to the very subtle differences as copolymer composition changed. For instance, fluorescence analysis of the copolymer films displayed a two stage hydration behaviour (similar to PNIPAm1), this two stage process being much more pronounced in the case of PNIPAm2 than for PEPm. We observed a greater degree of band separation for PEPm films at lower RH (than for PNIPAm2 films) indicating a more significant degree of polymer-probe H-bonding. At higher RH we see a reversal of this behaviour with PNIPAm2 now displaying a greater band separation. The greatly reduced band separation for PEPm coupled with its less pronounced two stage hydration behaviour indicated a greater water uptake ability of the PEPm films and a more uniform hydration process.

We observe similar effects as water is adsorbed by the NIPAM-co-EPM films as was observed for PNIPAm1 in that we noted a dramatic increase in the rate of change of $\log(I_{N^*/I_{T^*}})$, fluorescence intensity, $N^*$ bandwidth and band shifts indicating a larger change in local polarity and H-bonding of the probes with water. This results in
a greater dielectric stabilisation of the N* state compared to the T* state leading to an increase in the N* emission relative to the T* emission.

As EPM content was increased we observed a dramatic decrease in log($I_{N^*}/I_{T^*}$) values. These lower values indicate a reduction in local micropolarity as EPM content is increased (which is in line with solvatochromic results obtained in Chapter 4). This was also observed by Szczupak et al when studying copolymers of NIPAM-co-NtBA where the authors report a reduction in log($I_{N^*}/I_{T^*}$) values with increasing NtBA content and they attribute this to a decrease in local polarity. This was further evidenced by solvatochromic measurements where $E_T(30)$ values decrease as the hydrophobic NtBA content is increased.[21, 76, 88] The reduction we observed here in log($I_{N^*}/I_{T^*}$) values as EPM content is increased may indicate a dielectrically less stabilized N* state which favours the ESIPT T* state. But increasing humidity then results in the stabilisation of this N* state resulting in the increase of N* emission relative to T* emission caused by an increase in local polarity. As humidity increased we observed an overall larger increase in log($I_{N^*}/I_{T^*}$) values as EPM content was increased i.e. the overall increase in log($I_{N^*}/I_{T^*}$) values was greater than that observed for PNIPAm2.

We conclude that these 3-HF probes used in conjunction with a controlled humidity chamber offer considerable promise for studying in detail the hydration processes in thin thermoresponsive polymer films.
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