Fluorescence spectroscopy and imaging of dynamics and microstructure of acrylic polymer emulsions
Raja, T.N.

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Chapter 1

Fluorescence Spectroscopy in Polymer Science*

Abstract

Polymer science is an interdisciplinary field, combining chemistry, physics, and in some cases biology. Structure, morphology and dynamical phenomena in natural and synthetic polymers can be addressed using fluorescence spectroscopy. The most attractive aspect of fluorescent reporters is that their fluorescence parameters can give information on the nanometer length scale with an exceptional sensitivity, which allows data acquisition with submicrometer spatial resolution and millisecond time resolution. The use of fluorescent reporter molecules is, in principle, an invasive technique. Because of the large size of polymer molecules, however, small fluorescent reporter molecules of a length scale of < 2 nm can be considered a small perturbation.

Because of the enormous importance of synthetic polymers in our technology-based societies, almost every conceivable experimental technique has been applied in this field, but most of these tend to address the sample on a macroscopic scale. This chapter gives illustrative examples of the power of molecular fluorescence for investigating several microscopic aspects of polymer science.

1.1 Introduction

Paints comprise colorants (usually pigments), binders (often polymers) and a solvent. Water borne paints can be based on so-called latex materials, aqueous dispersions of polymer particles, which contain 40 - 50% of organic material and are made by emulsion polymerization. A typical recipe of emulsion polymerization comprises water, surfactant, water-soluble initiator, water insoluble monomers and additives. During emulsion polymerization micelles are formed when surfactant is added into water above the critical micelle concentration (CMC). The locus of polymerization is within these submicron micelles. Inside the cavity of micelles monomers undergo free radical polymerization in the presence of a water-soluble initiator and after polymerization it transforms into a latex particle, which can be regarded as a microreactor. One of the major advantages of emulsion polymerization is the excellent heat exchange due to the low viscosity of the continuous phase during the whole reaction. Emulsion polymers are widely used in the preparation of paints, coatings, paper, adhesives and many more.

Latex paints have gained intense attention due to their outstanding environmentally friendly nature. These materials have circumvented the extensive use of volatile organic compounds (VOC) ubiquitously present in large quantities in the solvent borne paints. According to the pollution prevention programme of European legislation, EU Directive 2004/42/EC, an organic compound having a boiling point lower than or equal to 250 °C at atmospheric pressure 101.3 kPa is classified as a VOC. The VOC limit for a waterborne paint was fixed to 30 g per litre in 2010 and this law was completely imposed in the same year.¹

The subject of the present Thesis is the study of film formation of waterborne latex using fluorescence spectroscopy. The use of fluorescence is not new in polymers; it was started with the pioneering work of Morawetz in the 1970s.²⁻⁴ Since then a number of issues in polymer science has been solved by using fluorescence methods. In one of his Science papers Morawetz discussed the use of fluorescence to investigate several features including conformational mobility of polymer chains, viscosity of polymers, glass transition phenomenon, polymer compatibility and the kinetics of diffusion controlled intermolecular polymer reactions.⁵

This Chapter presents an overview of the use of fluorescence spectroscopy to study polymers. Various types of solvatochromic, rigidochromic and fluorogenic fluorescent dyes can be used to probe numerous features such as polymerization mechanisms, kinetics, glass transition, film formation of latex emulsions and morphology, etcetera.
Due to the diversity, heterogeneity and complexity of polymeric materials it is very challenging for polymer scientists to investigate processes occurring at the molecular, microscopic level in these materials. While most polymeric materials are not fluorescent, they can be investigated by embedding fluorescent probe molecules that can be used to detect properties of their immediate surroundings, and dynamical processes that change the local environment on a variety of timescales. The combination of fluorescence techniques such as steady-state, and time-resolved fluorescence and fluorescence microscopy provides excellent opportunities to polymer scientist to investigate structure and dynamics in polymers. Some examples will be given in this chapter that deal with monitoring polymerization (kinetics and mechanism), the glass transition, film formation from latex materials and characterization of morphology etcetera.

We will first briefly discuss the typical fluorescence experiments and the information they can provide that is of importance for polymer science and then go into a number of areas in which these different experiments have been used. At the end of this Chapter an overview of the content of the Thesis will be given.

1.1.1 Steady-State Fluorescence

Steady-state fluorescence spectroscopy is relatively simple to perform and can provide significant information about the location and environment of a fluorescent probe. The shape of the spectrum (e.g. pyrene) and the position of the emission maximum (for solvatochromic probes) can provide information on local polarity and mobility. Energy transfer, as a measure of distance, between different fluorescent groups, can be detected from the emission spectrum. Excitation with polarized light combined with polarization-sensitive detection allows to measure anisotropy, a property that can give information on the rotational mobility of a probe molecule. The emission intensity can be used to obtain information on dynamical processes, using properly designed probes. For example, a probe molecule can be fluorogenic, the fluorescence being turned on when it is built into a polymer it can be rigidochromic or fluorescence can be quenched by diffusive processes. It is often difficult to quantify the emission intensity from a polymer sample because the absorption is usually not known, and samples are often scattering light. Ratiometric methods overcome this problem to a large extent. Dynamical processes are, generally, more accurately quantified using time-resolved spectroscopy.

1.1.2 Time-Resolved Fluorescence

The excited-state decay times of fluorescent molecules range from picoseconds to (many) nanoseconds. Dynamical processes that occur on these timescales can thus be monitored via time-resolved measurements. When dynamical quenching processes are of interest,
Chapter 1

time-resolved detection gives more reliable information than intensity measurements, which suffer from the uncertainty associated with light absorption versus scattering, which commonly occurs in polymer samples. Molecular reorientation, a measure of viscosity, is most directly measured via time-resolved anisotropy.\textsuperscript{23-24} In simple cases, the population of an excited state decays via first-order kinetics, that is, exponentially. In heterogeneous samples molecules in different environments decay with different time constants, which leads to a multi-exponential decay or to distributions of decay time constants. Medium reorganization on the timescale of the decay can lead to spectral evolution. In such cases it is helpful to include additional experimental variables, e.g. different excitation or emission wavelengths, to get a data set that can be subjected to global analysis.\textsuperscript{25-27}

1.1.3 Fluorescence Microscopy

In the past decades laser confocal fluorescence microscopy (LCFM) has emerged as a powerful technique to study the three-dimensional distribution of luminescent entities in biological and material sciences. In its basic form, LCFM offers a lateral diffraction limited imaging resolution of the order of a few hundred nanometers, and an axial resolution of one to several micrometers. In recent years, advanced techniques have been developed, and mainly applied to the life sciences, that allow sub-diffraction resolution imaging.\textsuperscript{28-30} LCFM can gain further resolving power by use of multiple detection channels for different colors or different polarization, or by including the fluorescence decay time as a variable (Fluorescence Lifetime Imaging (FLIM)).\textsuperscript{31} For thin films, for which depth-resolved information cannot be obtained anyway, direct 2D imaging (wide field) of the fluorescence intensities allows more rapid measurements, because scanning is not necessary. Fluorescence microscopy using a near-field approach offers higher resolution than LCFM, but only at the sample surface.\textsuperscript{32}

Fluorescence microscopy is the key to single molecule spectroscopy (SMS) because of its minimized detection volume, which allows discrimination of the emission of a single molecule from the background.\textsuperscript{33} SMS provides information on dynamics of individual molecules in heterogeneous environments. In contrast to ensemble measurements, which yield information on average properties, SMS provides information on rare events and gives access to distributions and time dependencies of properties of individual molecules. This technique can be applied to investigate various processes in polymer science such as complicated relaxation processes of polymer chains near the glass transition temperature in amorphous solids\textsuperscript{34-36} rotational diffusion of single molecules in polymers\textsuperscript{37} visualizing the dynamics of polymer chains and translational diffusion of monomers in polymerizing solution.\textsuperscript{38}
1.1.4 Types of Fluorescent Probes

In this chapter we will focus on fluorophores that are added as “molecular spies” to nonfluorescent samples. Polymers that are intrinsically fluorescent are outside the scope. In practice, polymer samples usually contain fluorescent impurities, which precludes the use of dyes absorbing in the UV (< 350 nm), unless special precautions are taken.

For some properties of interest, any dye with a suitable brightness and spectral properties can be used. If one is interested in properties related to diffusion, the molecular size is important. Energy transfer can be studied with appropriate pairs of energy donors and acceptors. Rigidochromic dyes are typically based on a nonradiative decay process that is suppressed when the molecule's environment is rigidified, giving emission of a photon a chance in competition with the radiationless excited state decay process. Re-complexes and substituted olefins are typical examples, but also other large-amplitude excited state processes involving electron transfer can be used.

In many cases, it is desirable to covalently attach the reporter dye to the polymer of interest. In that case, invariably some steps of organic synthesis are required. Most authors use dyes that are easily derived from commercially available ones, such as dansyl derivatives. On the other hand, commercially available dyes are normally not complicated to synthesize, so modification of the starting materials in order to arrive at a copolymerizable dye is usually not difficult.

Solvatochromic fluorescent probes are dyes of which the absorption or emission color is sensitive to solvent polarity. These are generally electron Donor-π-Acceptor molecules, which exhibit a relatively small dipole moment in the ground state and a larger dipole moment in excited state. Among the known solvatochromic compounds, the s-coupled donor-acceptor systems developed by Verhoeven and co-workers are still the most sensitive.

1.2 Monitoring Polymerization

Fluorescence methods can be fruitfully used to investigate various dynamical phenomena in polymer science such as different types of polymerization processes (including crosslinking and gel formation), irreversible film formation by the physical interpenetration and diffusion of polymer chains across interfaces, phase separation, and reversible transitions such as the glass transition. This section will focus on the monitoring of polymerization processes.

The polymerization process starts with monomers, which are often low molecular weight and low viscosity liquids, which undergo a polymerization reaction and are converted into
high molecular weight polymers, with a much higher viscosity. The polymerization process may take place through different mechanisms:

- chain-growth reaction: addition of unsaturated monomer at the end of a growing chain.
- step-growth: through a stepwise reaction between functional groups of monomers.

Step-growth polymers increase in molecular weight at a very slow rate at lower conversion and reach moderately high molecular weight only at very high conversion (> 95 %). The mechanism and kinetics of different types of polymerization are very different but a common feature is the increase in viscosity as polymerization proceeds, which can be effectively followed by fluorescent probes that are sensitive to the mobility and rigidity of the medium.

The increase in viscosity can be probed in different ways. Emission will be anisotropic when the molecular rotation of the probe molecule is hindered on the timescale of the excited state lifetime, typically several nanoseconds. Rigidochromic probes fluoresce weakly in a medium of low viscosity due to a radiationless decay process associated with a large-amplitude motion ("molecular rotor"), which is suppressed when viscosity is increased. Solvatochromic fluorescent probes are also sensitive to viscosity because the stabilization of the excited state dipole requires medium reorganization. Finally, diffusive quenching processes can be used to probe translational diffusion.

Pioneering work by Loutfy established 4-(N,N-dialkylamino)benzylidene malononitriles as prototypical rigidochromic probes. The fluorescence during homogeneous-phase polymerization of styrene or acrylates turns on rather abruptly when the free volume drops below a certain value (Figure 1). At low conversion, and at high conversion, these probes are not sensitive to the degree of polymerization.

![Figure 1](image-url)  
**Figure 1.** A) Prototypical "molecular rotor" 1; B) fluorescence intensity change as a function of styrene polymerization time.54
The rapid excited state decay through double bond rotation is related with the characteristic electronic structure, and is found in many Donor-\(\pi\)-Acceptor systems. This kind of fluorescent probe is intrinsically also solvatochromic, so the increase in viscosity is accompanied by a shift of the emission to shorter wavelengths. The solvatochromic effect is more pronounced in more extended Donor-\(\pi\)-Acceptor compounds.

Jager et al. monitored photopolymerization of dimethacrylates of various molecular sizes and polarities.

![Chart 1. Chemical structures of fluorescent probes used for monitoring the degree of (photo)polymerization of diacrylates.](image)

For all five solvatochromic fluorescent probes used in this study (Chart 1), the emission shifted towards shorter wavelengths upon polymerization because the dipolar excited states of the probe molecules are less stabilized in polymeric networks than in corresponding monomeric phases. Enhancement of fluorescence intensity was observed upon polymerization, which was most pronounced for 4-(dimethylamino)-4'-nitrostilbene.

The blue shift increases when monomers with shorter spacers were used, because this leads to a denser polymer network. This behavior was found in accordance with the fact that solvatochromic probes mainly monitor the changes in rigidity of the medium upon polymerization.

Exceptionally large fluorescence solvatochromic shifts are found in the sigma-coupled donor-acceptor systems investigated by Verhoeven and coworkers. The prototype 1-phenyl-4-(4-cyano-1-naphthylmethylene)piperidine (7) has a highly dipolar excited state with a dipole moment of 25 \((\pm 2)\) D. Depopulation of this dipolar excited state occurs via remarkably efficient radiative decay to the ground state, with quantum yields up to 85 \%. Because of these outstanding properties compound 7 was given the
nickname FluoroProbe.

Time-resolved spectral and polarization measurements were carried out to follow the dynamics of 7 during polymerization of methyl methacrylate (MMA). The emission maximum $\lambda_{\text{max}}$ of 7 in MMA is 565 nm. Curing of MMA was carried out at 85 °C for 15 h. Initially, a gradual hypsochromic shift occurred, but after ca. 4 h a jump of ca. 100 nm accompanied by an increase of fluorescence intensity was observed (Figure 2). The degree of fluorescence polarization was also measured, which served as a measure for the rotational mobility of the probe molecules. The anisotropy increased rather suddenly during the curing, but well before the large shift in the emission maximum occurred: anisotropy and emission maximum probe molecular rotation and medium relaxation, respectively. The anisotropy reaches its maximum when the rotation time of the probe molecule exceeds the excited state decay time, but the solvatochromic shift retains sensitivity to the extent of polymerization longer because it is caused by side chain rotations of the polar groups in the polymer.

![Chemical structure of 7](image)

Figure 2. A) Chemical structure of 1-phenyl-4-[(4-cyano-1-naphthyl)methylene]piperidine (Fluoroprobe) (7), B) Fluorescence maximum (open squares) of 7 in PMMA and fluorescence polarization (closed triangles) $p = (I_{h} - I_{v})/(I_{h} + I_{v})$ as a function of the thermal curing time at 85 °C. From reference 52.

For evaluation of heterogeneous systems, such as polymeric materials, where many species with different microenvironments are expected, steady-state spectroscopy has its limitations. Fluorescence lifetime as an additional observable can help to get a more accurate picture of such complicated structures. To show the combined power of steady-state and time-resolved fluorescence Fluoroprobe 7 was applied to study the structure of polystyrene (PS) latices and polystyrene-diglycidylmethacrylate (PS-GMA) core-shell latices. The emission maximum $\lambda_{\text{max}}$ of 7 in toluene is at 475 nm; in PS it is blue-shifted to 445 nm. In PS-GMA Fluoroprobe 7 exhibited three emission maxima at 380, 410 and
450 nm. Steady-state spectroscopy revealed that the 380 nm component is due to a decomposition product of 7 formed under the radical curing conditions. Time-resolved emission was applied to obtain a clear picture and explanation of the three emission maxima in the PS-GMA matrix. The lifetime distribution of 7 in the PS latex, quantified using the Maximum Entropy Method (MEM), is clearly different from that observed for the core-shell PS-GMA latex. It was noted that in high viscosity matrices the fluorescence lifetime depends on the emission wavelength. In addition the heterogeneity of the matrix adds to the complexity of the fluorescence lifetime data. Due to such complications it is not possible to use lifetime data in a quantitative way.

In the previous examples, the fluorescent probe molecule was simply mixed with the sample. The disadvantage of that approach is that the sensing molecule may accumulate in specific parts of the sample, e.g. in the monomer phase, and only report on those parts rather than on the sample as a whole. This problem can be solved to a large extent by introducing fluorogenic probes, of which the fluorescence is turned on only when they are covalently incorporated into the polymer. Warman et al. applied fluorogenic probes to study radiation-induced polymerization of MMA. Introduction of a maleimido moiety on Fluoroprobe 7 leads to the non-fluorescent “maleimidofluoroprobe” (MFP) 8. Removal of the double bond of the maleimide turns on the fluorescence of the chromophore (Scheme 1).

![Scheme 1. Fluorogenesis of maleimide substituted probe molecules. MFP 8 and MPy 9 are non-fluorescent. Removal of the double bond in the maleimide unit turns off a quenching pathway.](image)

Warman et al. monitored the course of radiation-induced polymerization of MMA in a cobalt gamma ray source by in situ fluorescence measurements. During polymerization of MMA a gradual increase in fluorescence intensity of copolymerized 8 was observed during the first 5 h. During this period the wavelength of emission remained almost constant,
indicating that there was no serious increase in the viscosity of the medium (Figure 3A). At close to 6 h elapsed time a dramatic increase in the intensity and decrease in the maximum wavelength took place, indicative of sudden rigidification of the matrix (Figure 3).

![Figure 3. A) Dependence on irradiation exposure time of the wavelength maximum (upper) and integrated intensity (lower) of the fluorescence from a methyl methacrylate solution of MFP 8; B) fluorescence intensities of MPy 9 and pyrene under the same conditions. From reference 66.](image)

Although the dual information provided by MFP 8 is useful, it is more difficult to extract a quantitative measure of the degree of polymerization from the data. That is more straightforward when the photophysics of the incorporated probe are unaffected by changes in viscosity or dielectric constant of the medium. This requirement is met by the pyrene derivative MPy 9. The fluorescence of the pyrene chromophore in MPy is completely quenched but it grows on irradiation of the MMA solution as the fluorogenic conversion of MPy occurs (scheme 1, Figure 3B). Interestingly, both MPy and pyrene show an increase in emission intensity when the gel point was reached.

Other pyrene derivatives also work as fluorogenic probes, e.g. N-(1-pyrene)methacrylamide, which was also applied for quantitative monitoring of radiation induced polymerization of MMA by Warman and co-workers67 or N-(2-anthracene)methacrylamide, which was recently used to monitor the kinetics of microemulsion polymerization.68
These examples clearly demonstrate that application of fluorogenic probes can determine concurrently both the degree of polymerization and the changes in viscoelastic properties of the medium as polymerization proceeds. The same concept has been used in the studies described in this Thesis (Chapter 2, 3 and 4). A fluorogenic fluorescent probe was covalently attached to the co-polymer during emulsion polymerization and photophysical properties of the probe were used to investigate the mobility, viscosity and polarity of the medium in bulk as well as in film formation.

Interestingly, Warman and co-workers recently used the fluorescence monitoring of polymerization as a tool for the detection of gamma-radiation.\(^{69}\)

Other phenomena that can be used to probe polymerization via the viscosity of the medium are those based on excited state interactions of fluorophores with other molecules. Excimer formation, energy transfer, and any quenching mechanism can be used. For example, Valdes-Aguilera et al.\(^{70}\) used the quenching of pyrene and its excimer by the initiator AIBN during the polymerization of MMA. The ratio of monomer to excimer emission increased during the process, because the quenching by AIBN affects mostly the excited monomer, which has a higher energy than the excimer and is much more reactive. The authors observed that the fluorescence ratio was a good measure of microviscosity around the fluorescent probe, which changes linearly with concentration up to 20 – 30 % polymer, but beyond a certain degree of polymerization, became independent of polymer molecular weight. Thus, the methods based on diffusive quenching are particularly suitable in the early stage of polymerization, where rigidochromic probes are insensitive and solvatochromic probes have a weak response (see Figure 4A). A correlation between the rate of polymerization determined gravimetrically and that of fluorescence is presented in Figure 4B. On the basis of this relationship, Valdes-Aguilera et al. concluded that the rate of increase in the fluorescence ratio is a direct measure of rate of polymerization.

A related application of pyrene was explored by Pekcan and co-workers. Pekcan and Kaya\(^{71}\) investigated the formation of gels when styrene in the presence of different concentrations of the cross-linker divinylbenzene (DVB) is subjected to Free-radical Crosslinking Copolymerization (FCC). Using excited state decay time measurements of pyrene they could monitor the consumption of styrene, which acts as an excited state quencher, in samples with various DVB content. In this way it could be shown that early gelation takes place at high DVB content, which leads to relatively large amounts of unreacted monomer. Slow polymerization on the other hand results in a larger increase in the decay time of excited pyrene because fewer monomers are trapped in the gel.
1.3 Physical Properties

1.3.1 Glass Transition

Among the physical characteristics of polymers, the glass transition temperature $T_g$ is one of the most important. Above the glass transition, in the rubbery state, the polymer has a larger free volume, the polymer chains are more mobile, and the material is softer than below $T_g$. Such differences in environmental properties can be probed e.g. with malachite green.$^{49}$ Like other properties, the glass transition changes when polymer films are made very thin, that is, when the thickness becomes comparable with the size of the molecules.$^{72-74}$ In the early 1990s seminal studies showed that confinement of amorphous materials at the nano-scale led to significant deviations of $T_g$ from the bulk values. Keddie et al. used ellipsometry to characterize $T_g$ by measuring film thickness as a function of temperature.$^{75}$ In 40 nm thick polystyrene (PS) film supported on a silica substrate a reduction in $T_g$ was observed. A 17 nm thick PS film exhibited a 21 K reduction of $T_g$ relative to the bulk value.

Ellison et al.$^{76}$ reported the first use of a fluorescence method to determine the effects of decreasing film thickness on $T_g$. They studied pyrene-doped polymer films of polystyrene (PS), poly(isobutyl methacrylate) (PiBMA) and poly(2-vinylpyridine) (P2VP) spin coated onto fused quartz.

Figure 4. A) Increase in the fluorescence ratio $R$ (= ratio of fluorescence intensity at 393 nm (pyrene monomer) to that at 476 nm (excimer)) during polymerization of MMA at 50 °C; B) correlation between the rate of increase in fluorescence ratio and the rate of polymerization measured gravimetrically. From reference 70.
Figure 5. Pyrene emission spectrum in a 350 nm thick polystyrene film at 398 K (bold curve) and 298 K (thin curve). From reference 76.

Figure 5 shows the effect of temperature on the fluorescence of pyrene doped at trace levels in a PS film. The fluorescence intensity is significantly reduced with increasing temperature. This is commonly observed with molecular fluorophores because rates of nonradiative decay increase with the thermal energy in the system. Importantly, the temperature dependence of the intensity is different in the glassy and the rubbery states. $T_g$ values were obtained from the intersection of the lines associated with the linear temperature dependences of the fluorescence intensities in the two states (Figure 6).

Figure 6. Fluorescence intensity as a function of temperature monitored at emission wavelengths of 395 nm (circles) and 384 nm (triangles). Pyrene-doped PS films were, A) 487 nm and B) 24 nm thick. Data were normalized to the intensity at 365 K and the 395 nm data were arbitrarily shifted vertically. From reference 76.

Figure 6 shows the temperature dependences of the emission intensities at two wavelengths (384 nm and 395 nm) for PS films on fused quartz that were 24 nm and 487 nm thick. These results were obtained by heating the polymer films well into the rubbery state and then measuring intensities during stepwise cooling. In the case of the 24 nm and 487 nm thick polystyrene samples, the apparent $T_g$ values were $362.6 \pm 2.2$ K and $372.8 \pm$
1.4 K and, respectively. The reduced $T_g$ of the ultrathin film was attributed to the weakness of the interactions between the substrate and PS.

![Graph](image)

Figure 7. Fluorescence intensity as a function of temperature monitored at an emission wavelength of 374 nm. Pyrene-doped P2VP films were 119 nm (circles) and 28 nm (triangles) thick; data normalized to 1 relative to intensity at 375 K and 400 K, respectively, and arbitrarily shifted vertically. From reference 76.

The temperature dependence of pyrene emission intensity for two P2VP films, one 24 nm and the other 119 nm thick, showed apparent $T_g$s at $398.0 \pm 0.9$ K and $379.3 \pm 1.4$ K, respectively. The bulk $T_g$ for P2VP was 372 K (see Figure 7). The rise of $T_g$ with decreasing thickness of film was attributed to the strong polymer-substrate interaction between the nitrogen atoms of the pyridine units of P2VP and the hydroxyl groups at the quartz surface.

The examples show that positive as well as negative deviations of the ultrathin film-$T_g$ from the bulk $T_g$ can occur, which can be attributed to the interaction with the surface.

In 2008, Kim et al. reported determinations of $T_g$ in freestanding single-layer polymer films using a self-referencing fluorescence intensity ratio method. The fluorescence spectral shape of co-polymerized 1-pyrenylmethylmethacrylate (MApyrene) was found to change as a function of temperature, and the changing intensity ratio of two vibronic bands could be used to characterize $T_g$. This $I_3/I_1$ ratio is well known to depend on solvent polarity. In this case, this “solvatochromic” effect was effective only when the pyrene unit was connected to the methacrylate with a short spacer. Figure 8 shows that temperature has a strong effect on the fluorescence spectral shape and intensity of MApyrene-labeled PS. Kim et al. concluded that freestanding films exhibit much stronger effects of confinement on $T_g$ than substrate-supported films. The self-referencing fluorescence method worked in polymers other than PS such as poly(isobutyl methacrylate) (PiBMA) and poly(butyl acrylate) PBA. In more recent studies, the confinement effect was shown to be strongly reduced when polymer films contained...
additives\textsuperscript{78} or when poly(vinylacetate) films were wet.\textsuperscript{79} A technical advancement was the use of fluorescence decay time instead of intensity.\textsuperscript{80}

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Figure 8. (left) Temperature dependence of fluorescence emission spectra of free standing films of MA-pyrene labeled PS, (right) temperature and thickness dependences of the ratio of intensities at the third and first peaks of free standing MA-pyrene labeled PS films; 190 nm thick film (circles) and 50 nm thick films (squares).\textsuperscript{77}

1.3.2 Other Mechanical Properties Probed by Solvatochromic Probes: Morphology

By using steady-state fluorescence, thermal and mechanical properties of block copolymers doped with \textit{FluoroProbe} \textsuperscript{7} were investigated by Hofstraat et al.\textsuperscript{81} They studied block copolyether-polyesters, composed of alternating blocks of polybutylene terephthalate (PBT) and polyethylene glycol (PEG) composed of 70 wt % PBT ($M_w \, 9000$) and 30 wt % PEG ($M_w \, 4000$). \textit{FluoroProbe} \textsuperscript{7} was introduced into the copolymer by swelling polymeric sheets or threads in dichloromethane solutions of \textit{FluoroProbe}. Fluorescence spectra of \textsuperscript{7} in homopolymers of PBT and PEG and in block copolymer PBT/PEG at room temperature are shown in Figure 9.

The building blocks have rather different physical properties: PBT is hydrophobic, rigid and semicrystalline, but PEG is hydrophilic and flexible. In the PEG 4000 homopolymer, the emission maximum was at 515 nm, but in PBT it was red-shifted to 540 nm. This unexpected result showed that a mobile environment exists in PBT, in which the polar ester groups can stabilize the highly dipolar excited state of \textsuperscript{7}. Interestingly, an even more mobile environment seemed to be present in the PBT/PEG copolymer thread than in the homopolymers, as indicated by the $\lambda_{\text{max}}$ of 560 nm. The emission of \textsuperscript{7} in copolymer sheets, on the other hand, was strongly blue shifted (Figure 10). This was taken as an indication
that processing of the copolymers into sheets leads to orientation and rigidification of parts of the amorphous material.

![Graph](image1)

**Figure 9.** Emission spectra of $\lambda$ in PBT 25000 homopolymer, PEG 4000 homopolymer, PBT/PEG block copolymeric sheet, and PBT/PEG block copolymeric thread. From reference 81.

In stretching experiments the isotropic thread showed a monotonous blue shift upon extension. This was attributed to increasing orientation of the polymer chains, which results in a reduction of mobility of the environment of the probe molecule. The fluorescence of $\lambda$ in the sheet initially showed a bathochromic shift (decreased order) upon stretching, but after 50 % stretch a hypsochromic shift set in, much like in the thread.

![Graph](image2)

**Figure 10.** Response of the $\lambda_{max}$ of $\lambda$ to stretching of the PBT/PEG copolymeric thread and sheet.81

Figure 11 shows the effects of temperature on the emission maximum, together with the specific heat (Differential Scanning Calorimetry). Going from -50 to 0 °C the mobility of
the matrix increases, leading to a red shift of the emission maximum of 7. The red shift accelerated at 0 °C due to melting of PEG blocks (confirmed by DSC and TMA). The blue shift encountered between 170 and 200 °C was attributed to premelting of small PBT crystals. The fluorescence data of the incorporated probe molecule nicely reflect the three transitions occurring in the copolymer in this temperature range.

![Figure 11. Differential Scanning Calorimetry and temperature dependence of λmax of 7 in PBT/PEG copolymer. From reference 81.](image)

### 1.3.3 Mechanical Properties: From Latex To Coatings

Waterborne aqueous polymer dispersions are increasingly important in numerous applications, e.g. as paints and adhesives. Environmental concern is a major driving force for the replacement of solventborne coatings. Film formation from e.g. latex materials created by emulsion polymerization is not a trivial matter. It requires a low $T_g$ for the particles to merge after application of the film on a substrate and evaporation of water. In order to achieve this, organic co-solvents are added, which is an undesirable practice that needs to be minimized.

Swelling of the polymer particles in waterborne latex materials by these so-called coalescing agents was observed by Raja et al. using a fluorogenic and solvatochromic probe molecule similar to 8. The emission wavelength could be correlated with the concentration of co-solvent, which forms the basis for a method to follow the kinetics of redistribution of co-solvents when different latices are mixed.

The mechanism of film formation from waterborne organic coatings is an important issue. Film formation is thought to take place in three or four steps. After application of an aqueous dispersion on a substrate water evaporates and the spherical latex particles come into an irreversible contact. In the next stage the voids between the spheres are filled...
by the deshaping of particles to polyhedra. Finally, coalescence of particles followed by chain diffusion and entanglement lead to the formation of a coherent film.

![Figure 12](image)

**Figure 12.** A) Effect of co-solvent DPnB on the emission of copolymerized probe; B) dependence on the position of the emission maximum on the amount of co-solvent for different polymers.¹⁹

Fluorescence resonance energy transfer (FRET) is a versatile approach to investigate coalescence and interdiffusion in polymers.⁸⁸ In FRET two kinds of fluorescent dyes are used, one is excited and acts as energy donor and the other is the acceptor. For investigation of coalescence or diffusion during latex film formation equivalent amounts of latex particles are labeled with each of the dyes. When the particles are intact, the distance between the two dyes is large enough that energy transfer does not occur. However, when the film is formed donor and acceptor labeled latex particles come into close contact, and the dyes approach each other to a distance that is small enough for energy transfer to take place. Therefore FRET provides a clear indication of coalescence and interdiffusion of latex particles during film formation.⁸⁸

In 1990 Winnik and coworkers⁸⁹ reported the first experiments on nonradative energy transfer to study polymer diffusion across the particle boundary during latex film formation. PMMA particles of 1 µm diameter, of which one batch was labeled with phenanthrene and the other with anthracene, were mixed, spread as a thin film and annealed above the glass transition temperature of PMMA. Energy transfer measurements effectively followed polymer diffusion across the particle boundaries during drying of the latex film. They derived a diffusion coefficient $D$, which characterized polymer diffusion. For PMMA particles at temperature between 403 and 450 K, diffusion coefficient values ranged from $6 \times 10^{-18}$ to $9 \times 10^{-14}$ cm² s⁻¹.

More recently, interdiffusion of polymers in latex film formation was studied in real time.¹⁵ Haley et al. employed time-resolved fluorescence to measure donor fluorescence decay from 0.5 mm diameter spots at various positions in a drying film. It is well known that drying of latex films takes place from the edges of the film inward. The film drying
process was temporarily arrested by sealing the film in an airtight chamber, and reducing the sample temperature to near or below the glass transition temperature $T_g$, which stopped polymer diffusion. Latex particles with a low $T_g$ were composed of butyl acrylate : methyl methacrylate : methacrylic acid in 50 : 49 : 1 ratio. The donor fluorescence decay was measured at various positions across the arrested latex film. The extent of polymer diffusion was demonstrated as a function of distance from the wet-dry edge in the latex film. Energy transfer efficiency revealed a rapid evolution of film structure. Subsequently, the new technique was applied in a number of case studies of film formation at ambient temperature.\(^9\)

Turshatov et al.\(^9\) employed time-resolved fluorescence spectroscopy to monitor intermixing of polymer particles during film formation of labeled poly(methylmethacrylate). Drying of the films was monitored by the efficiency of energy transfer between the phenanthrene donor derived from monomer 10 and non-fluorescent nitrophenylpyrrolidine acceptor (monomer 11) (Figure 13), as well as the scattering intensity and the lifetimes of donor units. Scattering can provide valuable information when analyzing the drying of the latex film: the disappearance of scattering marks the end of the compactification stage. The changes of the donor lifetime over time closely correlate with the changes of scattering intensity. When the interstitial water voids disappear, the light scattering also disappears, and the donor lifetime shows a small but sharp decrease.

On the basis of the FRET experiments Turshatov et al. concluded that a well-intermixed layer of 20 nm develops at the interparticle junction soon after particles come into contact. This does not develop further, and thermal annealing is needed to bring about further interdiffusion. Interparticle cohesion as reflected by FRET efficiency frequently develops before the interstitial stage disappears. These findings prove that the neighboring polymer spheres develop an intermixed layer after they come into contact.

![Figure 13. Chemical structures of monomer donor and acceptor dyes used in reference 91: donor phenanthrene methacrylate, Phe-MA 10, acceptor ([1-(4-nitrophenyl)-2-pyrrolidinemethyl]acrylate, NPP-A) 11.](image)
1.4 Microscopy: Single Molecule Studies And Imaging

Confocal microscopy has become a very popular tool for investigating many processes in biological, medical and material science. The application of a wide variety of fluorescent molecules made it possible to tag the specific position in the sample and study components and processes with sub-micrometer resolution. It is also possible to investigate phenomena at the single molecule level by using confocal microscopy. The main advantage of confocal microscopy is that it offers to collect serial optical sections (0.5 to 1.5 µm) from thick specimens up to 50 µm.

Single molecule fluorescence techniques (SMD) offer exciting new possibilities for the study of polymer dynamics. Individual fluorescent molecules show the same observables as can be measured on an ensemble of molecules, such as spectrum, polarization and decay time. SMD allows determining the distributions of these properties, but more importantly, fluctuations of the properties can be monitored on time scales from milliseconds to hours. For example, fluctuations of the emission rate of very efficient emitters could be correlated with fluctuations of the local density, in other words, the free volume, in polystyrene films well below \( T_g \)\(^{94, 95} \). Molecules embedded in polymer films show molecular reorientation jump on timescales of seconds and longer at temperatures near and above \( T_g \)\(^{96} \). These can be observed by measuring the polarization of the emitted light, but more elegantly by wide field imaging techniques, which allow direct determination of the direction of the transition dipole moments. Zheng et al.\(^{98} \) recently applied this approach to demonstrate the rotational motion of dye molecules covalently attached to PS in very thin films. Diffusive motions can be observed with nanometer spatial resolution, which enables optical microrheology. A combination of fluorescence correlation spectroscopy and wide field imaging has been used to monitor different stages of polymerization based on the mobility of copolymerizing probe molecules.\(^{38} \)

In the above-mentioned examples, the molecules used should be bright and photostable, and preferably not do anything other than absorb and emit light. More complicated systems, for example showing photoinduced electron transfer, can have additional value. Hofkens and co-workers measured the fluctuating intramolecular electron transfer of donor-substituted perylene imides to obtain information on fluctuations of the polymer free volume in PS near ambient temperature.\(^{100} \)

Siekierzycka et al.\(^{36} \) showed that the fluorescence of the perylene bisimide 12 could be switched off and on reversibly when heating and cooling films across the glass transition. Interestingly, the control of photoinduced electron transfer in this case is not related to stabilization of the charge-transfer state by dipolar groups in the medium, but by the kinetic limitations imposed by the lack of free volume (Figure 14).
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A)

B)

Figure 14. A) Chemical structure of 12, a perylene bisimide substituted with calix[4]arene, B) number of fluorescing molecules of 12 detected in a 26 µm × 26 µm area in a poly(vinylacetate) (pvac) matrix (left axis) as a function of temperature and part of the DSC curve (right axis) with the determined $T_g$ of the polymer. From reference 36.

Confocal microscopy as an imaging technique began to attract the interest of materials scientists in the 1990s and continues to be used on a modest scale. The polymer field so far has largely failed to pick up the spectacular developments in microscopy techniques in the past decade. There is a great potential here.

Blending of different polymers is a way to create new physical properties using existing materials. Most polymers, however, are poorly miscible, and phase separation commonly occurs. The properties will then be dependent on the spatial arrangement of the different phases, in other words, the morphology. In an early application of confocal microscopy, a depth resolved study of films formed from a mixture of PMMA and polystyrene was carried out by Li et al.\textsuperscript{101} They prepared a blend of PMMA labeled with the dye N-methyl-N-(4-(7-nitrobenzo-2-oxa-1,3-diazole))-2-aminoethylmethacrylate (NBD-MMA) with PS. Films were prepared by solvent casting and examined with a confocal fluorescence microscope (see Figure 15). Different morphologies of the film were obtained; a surface slice of 0.5 µm thick at the air-film interface showed a uniform size of PMMA spheres of 5-6 µm with a remarkable periodicity. In a slice of the same film 3 µm below the surface PMMA particles were present in larger as well as smaller sizes. On a spatial scale of 1-2 µm a sharp transition was observed between surface morphology and the bulk morphology of the system. A further slice of 6 µm beneath the surface showed a higher content of smaller droplets with a large size dispersion. These were supposed to have arisen from phase separation through a nucleation and growth mechanism, but the regular pattern of PMMA-rich droplets on the surface could not be explained.
Figure 15. Laser confocal microscopy images of solvent cast films (from toluene) comprised of 10 wt % of PMMA labeled with the dye NBD and 90 % polystyrene. The left image is the film surface, the middle image is a slice of 3 µm beneath the surface and the right image is of a slice 6 µm beneath the surface. From reference 101.

Winnik and co-workers continued to use confocal microscopy over the years.102-104 Recently, they investigated the morphology and miscibility of blends of thermoplastic olefins (TPO), of practical importance for the automotive industry.\textsuperscript{105} TPO are blends of polypropylene and impact modifiers, in this case ethylene–butene rubber (EBR) copolymers. Being very hydrophobic, TPO are difficult to paint. Therefore chlorinated polyolefins (CPO) are used as adhesion promoters. Laser scanning confocal microscopy was used to image two of the three components of the blend. The CPO component was labeled with a coumarine, excited at 351 nm, the EBR with a benzothioxanthene fluorophore (HY), excited at 488 nm. The blends were prepared by precipitation of a 1 : 1 : 1 mixture of the three polymers from solution, and annealed at a temperature (180 °C) where the polymers are molten. The images (Figure 16) show convincingly that EBR and CPO mostly localize together, forming domains within the isotactic polypropylene (iPP) material. The CPO adhesion promoter, however, is slightly miscible with iPP. In this study, other techniques were used to obtain information on the miscibility of the components, which led to a consistent picture. Of all the methods used, confocal microscopy, however, gave the most direct and visual insight into the system. The incorporation of the fluorescent labels was considered to be only a small perturbation. As a check, it was shown that the labeled polymers were fully miscible with the non-labeled ones.
1.5 Conclusion

As illustrated by the examples in this chapter, fluorescence techniques have been applied successfully to polymer science over the past three decades. In this field, fluorescence is only one of the many research tools that are used. In the life sciences, on the other hand, fluorescence imaging is a major technique because of the essential role of sub-micrometer three-dimensional structure in the cell. In our opinion, there is plenty of opportunity to take advantage of the rapid progress in fluorescence methods in the life sciences by applying the functional dyes developed there as well as the sophisticated modern microspectroscopic techniques to polymer science. Many of the commercially available dyes can be readily adapted for use in polymers, or even used directly. To modify dyes with linkers that can be co-polymerized for covalent inclusion in polymers is not more difficult than to attach anchors for biological labeling. Although the questions in polymer science are different from those in life science, the use of long-wavelength excitation\(^{106}\) (possibly using two photon absorption) will have advantages in the former field as well,
due to the tendency of heterogeneous materials to scatter light.

Molecular fluorescent probes can give information on the dynamics of their environment on timescales from picoseconds (solvent relaxation) up to months or years (physical aging). Fluorescence-based imaging gives access to 3D-structure and morphology information with a potential resolution down to a few tens of nanometers. These methods have great potential for polymer science. Given the present disciplinary structure of science, however, the full realization of this potential will require more collaboration of scientists from the communities of molecular fluorescence and polymer physics and chemistry.

1.6 Scope of this thesis

This Thesis encompasses the research work on the investigations of film formation from latex dispersions by using fluorescence spectroscopy. In continuation of film formation several experiments have been performed in the bulk to get insight in the interaction of organic solvents (co-solvents) with polymer particles. These studies have been carried out on hydrophobic and hydrophilic acrylic latex emulsions, prepared via seeded emulsion polymerization in low and high $T_g$ combinations. Throughout this Thesis several fluorescent molecules have been used as fluorescent spies, some covalently linked to the polymer backbone, some only physically attached to the polymer particles. The photophysical properties of these fluorescent molecules have disclosed dynamics occurring in real time.

Chapter 2 describes the use of an environmentally sensitive fluorescent probe covalently linked to the co-polymer in the latices. It proved an efficient indicator of the presence of co-solvent in the polymer particles. These investigations were performed in the bulk latices and fluorescence emission quantitatively estimated the presence of co-solvent in the latex particles.

Chapters 3 and 4 describe the partitioning behavior of organic co-solvents in the latex emulsions. Migration of co-solvent between particles of the same latex material is discussed in Chapter 3. The experiments reported here concurrently describe the entrance of co-solvents into the latex particles and their escape from them. These studies show that the rate of co-solvent repartitioning in the low $T_g$ latices was very high (< 1 min). In high $T_g$ hydrophilic latices it took < 1 h to reach equilibrium. The migration behavior of co-solvent during blend formation when different kinds of latices with respect to the co-polymer and $T_g$ were mixed is the subject of Chapter 4.

In Chapter 5 the development of a methodology to study latex film formation using
confocal microscopy is discussed. A fluorescent dye physically absorbed to the co-polymer was employed in these experiments. Particular attention was paid to view an individual latex particle in the confocal images and with respect to that the concentration of fluorescent dye was optimized. For a drying film one-dimensional scanning provided the thickness of the film. Two and three-dimensional (2D and 3D) modes of confocal imaging were performed to view the individual latex particles at ambient temperature and in the presence of co-solvent.

Chapter 6 is about the application of the methodology of studying film formation using confocal microscopy described in Chapter 5. A perylene imide based highly stable and strongly fluorescent emitter was co-polymerized in the latex via emulsion polymerization. Co-solvent and annealing were applied to achieve film formation and tested the proof of working principle.

In Chapter 7 the preliminary experiments on latex film formation by using steady-state and time-resolved fluorescence are presented. Film drying as a function of time was studied at ambient temperature. Emission intensity and wavelength can be used to monitor the film drying process. Results obtained from these experiments were unexpected and ambiguous: a decrease in the emission intensity was always observed during the film drying.

### 1.7 References


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