Fluorescence spectroscopy and imaging of dynamics and microstructure of acrylic polymer emulsions
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Chapter 7

Fluorescence of Drying Latex Films

Abstract

Dye labeled latex films were investigated during drying using steady-state and time-resolved fluorescence spectroscopy. Unexpectedly, the emission decreased drastically with the passage of time, both with the solvatochromic probe MFT and with the inert Perylene Red dye. Many experiments were performed to resolve this issue such as film drying in nitrogen environment, functionalization of substrate, use of additive free latex, cleaning of the latex by dialysis, etcetera, but the reason for the decrease in intensity remained unclear. Removal of water-soluble components from the latex by dialysis led to slower decrease of fluorescence, so it is likely that water-soluble quenchers play an important role.

The shift of emission of the solvatochromic probe, however, was hypsochromic when film was dry, as expected, because the medium cannot relax on the short time scale of the excited state lifetime.
7.1 Introduction

Fluorescence spectroscopy has been found to be a useful tool to understand various phenomena in polymer science. Steady-state\(^1\) and time-resolved fluorescence\(^3\) have been extensively used for the characterization of various polymer properties and processes. The selection of an appropriate fluorescent molecule for an investigation is an important step. The mobility and viscosity of the medium can be efficiently detected by a mobility sensitive probe. Similarly, a solvatochromic probe is the first choice for the study of a system influenced by solvents.\(^5\)\(^,\)\(^6\)

The photophysical properties of the fluorescent molecules provide valuable information about the local environment. The steady-state fluorescence spectrum can reveal information about the mobility, viscosity, rigidity, and polarity of the local medium of the fluorescent molecule. Time-resolved fluorescence is very helpful in understanding the dynamical processes occurring on the time scales of picoseconds to microseconds. Heterogeneity of the sample can be detected by this technique, as it can lead to multiexponential decay of the fluorescence.\(^7\)

Latex film formation is a complex and multistage process. Stages of film formation have been studied using fluorescence\(^8\) and other methods.\(^9\) Our approach is to investigate the film formation stages using steady-state and time-resolved fluorescence. By utilizing these techniques monitoring of latex film drying can be performed as a function of time, temperature and co-solvent. Increase in viscosity occurs when water evaporates and latex particles concentrate. At this point the use of a mobility sensitive dye can reveal significant information about the viscosity of the medium.

As in Chapters 2 – 4 we used the solvatochromic fluorescent probe nicknamed Maleimidofluorotrope (MFT)\(^6\) \(^1\) covalently attached to the copolymer in the latices. Two other fluorescent dyes, which were added to the latex and have no covalent linkage to the polymer, were also used. These are MFT/n-butylamine adduct (2) a model compound of \(^1\) and Perylene Red (3) (Figure 1). Compound 1 was present in 6 ppm in low and high \(T_g\) hydrophobic and hydrophilic latices. In low \(T_g\) hydrophilic latex two batches of emulsions were prepared, one having 6 ppm and the other 18 ppm copolymerized dye \(^1\) in the latex. Latices labeled with 10 ppm of 2 and 3 were prepared by dissolving the probes in a co-solvent and mixing that with the latex. The excitation of 1 and 2 was carried out at 380 nm and detection of emission was in the range of 400 – 600 nm. Excitation and emission wavelengths for compound 3 were 560 and 580 – 800 nm, respectively.
Fluorescence of Drying Latex Films

Figure 1. Fluorescent probes used to label latices in film drying experiments. Maleimidofluorotrope (MFT) (1) shown as it was copolymerized in the latex. MFT/n-butylamine adduct (2) and Perylene Red (3), were added to the latex after emulsion polymerization having no covalent link to the polymer.

Latex materials used in the film formation studies were the low and high $T_g$ hydrophobic and hydrophilic acrylic latices that were described and studied in previous chapters. The preparation of these latices is described in Chapter 2. The co-solvents used in this chapter were 2,2,4-trimethyl-1,3-pentanediol mono(2-methyl propanoate) (Texanol) and di(propylene glycol) n-butyl ether (DPnB) having 2 and 5 % water solubility, respectively.

This Chapter is divided in two main parts: one deals with the film drying experiments performed with the steady-state spectroscopy and the other with the time-resolved fluorescence.

### 7.2 Film Drying on Steady-State Fluorescence

For all experiments described in this Chapter the method of casting films was the same, i.e., with the metallic bar that produces a film of a specific thickness (see Figure 2 in Chapter 5). The channels present on the surface of this bar control the thickness of the film. In most of the experiments we cast the film with a metallic bar that provides a thickness of 150 $\mu$m in the wet and 60 $\mu$m in the dry state. In some cases 40 $\mu$m wet and 15 $\mu$m dry film was studied. The substrate was a glass cover slip of 60 $\times$ 24 mm. In order to get a smooth and homogeneous film latices were formulated with co-solvents such as
DPnB and Texanol. The formulated latex was used to cast film for monitoring of film drying. After casting the fresh film it was placed in a chamber attached to the spectrofluorometer to study film drying. This chamber was equipped with an optical fiber attached to the spectrofluorometer. Its function was to bring excitation light from the lamp, and to collect the emission from the sample film and bring it to the detector. The film was placed horizontally on a sample holder and the optical fiber was adjusted to excite the film either from the top or from below. Figure 2A and 2B show the excitation carried from top or from bottom using the optical fiber.

![Diagram](image)

Figure 2. Schematic presentation of optical fiber exciting the horizontally placed sample latex film: A) from the top, B) from below.

### 7.2.1 Low $T_g$ Latex

Film drying of low $T_g$ hydrophobic latex was investigated by means of steady-state fluorescence. S/EHA20 latex was formulated with 9 % w/w DPnB and 9 % w/w Texanol co-solvents. The label was Maleimidofluorotrope 1 copolymerized in the latex. The concentration of this dye was 18 ppm in this latex. Films were cast by the metallic bar, which produced 150 and 60 μm thick film in wet and dry states, respectively. After casting fresh film it was immediately placed in the chamber attached to the spectrofluorometer equipped with an optical fiber for excitation and collection of emission from the sample. Excitation was done from the top of the film (Figure 2A). The excitation wavelength was 380 nm and emission was collected in the range of 410 – 600 nm. Fresh films were turbid due to the scattering of incident light, but they quickly become transparent after evaporation of water from the film. Drying of films was measured for 1 hour. The duration of measurement of one spectrum was 95 s and 21 spectra were recorded during one hour.

Figure 3 presents the spectra of 9 % w/w DPnB and Texanol formulated latices S/EHA20 films. The first curves in Figure 3A and 3B show the emission of fresh wet films and it was observed that the emission intensity decreased during film drying. In Figure 3B the drying
profile of Texanol formulated latex film followed the similar trend as shown with the help of arrows in Figure 3A. The shapes of the spectra were modeled as skewed Gaussian to obtain the emission intensity, emission maximum, width and skewness (Equation 1).

\[ I(\lambda) = I_0 + I_{\text{max}} \exp\left(-\ln2\left[\ln(1+2s(\lambda - \lambda_{\text{max}})w)/s\right]^2\right) \]

(1)

Figure 3. Fluorescence emission spectra of drying films of low \( T_g \) 1-copolymerized S/EHA20 formulated with A) 9 % w/w DPnB, B) 9 % w/w Texanol. \( \lambda_{\text{exc}} = 380 \text{ nm} \). Excitation was from the top.

Values of emission maximum, intensity, width and skewness of the peaks obtained from the fits were plotted against time and are presented in Figure 4.

A drastic decrease in the emission intensity was observed as a function of time during film drying (Figure 4A and 4B). This was unexpected because the amount of dye in the illuminated spot is not expected to change. A striking feature was the sudden but temporary rise of the emission intensity at ca. 20 min. This dramatic increase must be related to the loss of water from the samples, which is almost complete at this time. During film drying the emission maximum first shifted towards longer wavelength. Then it moved erratically back and forth but after 1 h in comparatively dry film it exhibited a sharp hypsochromic shift. In DPnB and Texanol formulated fresh films the emission maximum was at 450 nm and after an hour of drying the films showed emission at 443 and 448 nm, respectively.
Figure 4. Emission maximum, intensity, width and skewness plotted against time for 1-copolymerized S/EHA20 latex films formulated with 9 % w/w: A, C, E, G) DPnB, B, D, F, I) Texanol. Excitation and detection was from the top.
7.2.1.1 Low $T_g$ Latex Films in Nitrogen

The emission of 1 might be quenched by the presence of oxygen.\textsuperscript{10, 11} To see the effect of oxygen on the fluorescence of drying latex films an experiment was performed in which same fluorescent latex film was monitored in nitrogen and oxygen (air) environments separately.

Figure 5 shows the emission spectra of S/EHA20 formulated with 9 % w/w DPnB measured in air and in nitrogen atmosphere. Films were cast by the metallic bar that produced 40 and 15 $\mu$m thick film in wet and dry states, respectively. For oxygen no change in the experimental set up was made and experiment was performed on ambient condition. For nitrogen atmosphere nitrogen gas was continuously flushed through the chamber where the film was placed for drying.

Figure 5. Fluorescence emission spectra of drying films of low $T_g$ 1-copolymerized S/EHA20 formulated with 9 % w/w DPnB: A) in air, arrow a = fresh film, b = 1 week later, B) normalized version of (A), B) in nitrogen, arrow a = fresh film, b = 60 min later C) excluding the first spectrum of (B). $\lambda_{ex} = 380$ nm.

Both experiments showed emission of drying films decreased and no significant difference in case of nitrogen was observed. The decrease in fluorescence of film measured in air was

\[ \text{Intensity} \]
gradual and in nitrogen it showed a sharp decrease in intensity after first measurement. The large difference in the intensity of first and second emission spectra might be due to the faster drying due to the flushing of nitrogen in the chamber of spectrofluorometer. Moreover a thinner film was used than in the experiments described in Section 7.2.1.

7.3 High $T_g$ Latex Films

High $T_g$ hydrophobic and hydrophilic acrylic latices were measured in both ways: excitation light from the top (Figure 2A) and from below the film (Figure 2B). This was a test to see whether the way of film excitation has any effect on the emission of the drying films.

7.3.1 Excitation From Above

Fluorescence of high $T_g$ hydrophobic and hydrophilic latices was measured. S/EHA60 and MMA/EA60 latices were copolymerized with fluorescent dye 1 and the concentration of dye was 6 ppm in these latices. Excitation of films was carried out from the top of the film such that optical fiber was ca. 1 centimeter above the film (Figure 2A). This experiment was performed in ambient conditions (23 °C was the room temperature on the day of measurement). Thicknesses of wet and dry films were ca. 150 and 60 µm, respectively. Figure 6 presents the fluorescence of drying films of S/EHA60 and MMA/EA60 latices formulated with 9% w/w co-solvents DPnB and Texanol, respectively. Each measurement took 95 s and first 60 scans were automatically measured in 95 min. First 60 spectra were measured while exciting the film on the same area. Films were then removed from the instrument and remeasured after 24 h and 10 weeks. Once the film was removed form the instrument the position of measurement in the film was changed. As in the case of the low $T_g$ latices, we observed a decrease in the fluorescence during film drying in these high $T_g$ latices. As seen in Figure 6 the shape of emission bands in S/EHA latex film was not constant but in the MMA/EA film the shape of the band in early measurements was similar. The decrease in the emission of both latices was a common observation. Remarkably, it was relatively slow in the beginning in the case of the MMA/EA/DPnB sample.
Figure 6. Fluorescence emission spectra of drying films of high $T_g$ 1-copolymerized latices: A) S/EHA60 latex formulated with 9 % w/w DPNB, arrows: (a) = fresh film, (b) = 2 days old film, (c) = 10 weeks old film, (d) = 60 min old film B) S/EHA60 latices formulated with 9 % w/w Texanol, C) MMA/EA60 latices formulated with 9 % w/w DPNB, arrows: (a) = fresh film, (b) = 24 min old film, (c) = 2 days old film, (d) = 10 weeks old film. Fluorescence was measured in ambient conditions. $\lambda_{exc} = 380$ nm. Excitation was from above.

Plots of emission maxima, intensities, widths and skewnesses versus time more clearly represent these results. In Figure 7 the plots of S/EHA and MMA/EA high $T_g$ latices with DPNB are shown. In first 60 measurements the emission decreased but in later days it was higher than the last measurement of the 60 scans. This was more pronounced in S/EHA than in MMA/EA latex. The emission maxima of S/EHA60 in fresh and 10 weeks old film were observed at 455 and 449 nm, respectively, i.e., a 6 nm hypsochromic shift. The width and skewness of peaks decreased during drying. In MMA/EA60 fresh and 10 weeks old film emission maxima were observed at 477 and 463 nm, respectively. There was a hypsochromic shift of 14 nm. Like in S/EHA a decrease in the width and skewness of peaks was observed in the case of MMA/EA. In both DPNB and Texanol formulated films of hydrophobic and hydrophilic latices results were similar, i.e., a blue shift of the emission maximum and a decrease in intensity of dry films. Changes in the shape of the band, however, occur in ways that we cannot rationalize at this moment.
Figure 7. Fluorescence of hydrophobic and hydrophilic latices: Emission maximum, intensity, width and skewness plotted against time of latex films of: A, C, E, G) S/EHA60 formulated with 9 % w/w DPnB. B, D, F, H) MMA/EA60 formulated with 9 % w/w DPnB. Excitation was from the top.
7.3.2 Excitation From Below

Fluorescence of 1-copolymerized S/EHA60 latex formulated with DPnB and Texanol were measured on the steady-state fluorescence (Figure 8) with excitation from below (Figure 2B). Wet film thickness was 150 μm. Each measurement was carried out in 57 s and 60 measurements were performed, which took 57 min. First 57 measurements were automatically measured on the instrument and then films were removed and measured again after one week on another position in the film.

![Fluorescence emission spectra](image)

Figure 8. Fluorescence emission spectra of drying films of high Tg 1-copolymerized S/EHA60 formulated with: A) 9 % w/w DPnB, B) 9 % w/w Texanol. Fluorescence was measured in ambient conditions. \( \lambda_{exc} = 380 \) nm. Excitation was from the bottom through the cover slip.

Figure 9 presents the plots of emission maximum, intensity, width and skewness against time. These results are in many respects similar to those when the films were excited from the top. A decrease in the emission intensity was still observed in both films. The transient feature around 20 min observed in the low Tg hydrophobic latices (Figure 4) was clearly observed for the high Tg latex formulated with the Texanol but much less with the DPnB co-solvent.

In DPnB formulated film the emission maximum of the fresh film was observed at 451 nm and in further measurements it was blue shifted to 446 nm. However, 1 week later it unexpectedly was at 453 nm in the same film (Figure 9C). A similar trend was observed for the width. In the fresh film it was 97 nm and it decreased in initial 30 measurements. After that it started to increase and in the 1 week old film it was at 122 nm (Figure 9E). Skewness decreased from 0.37 to 0.33 in fresh and 1 week old film, respectively (Figure 9G).
Figure 9. Fluorescence of hydrophobic and hydrophilic latices: emission maximum, intensity, width and skewness plotted against time for S/EHA60 latex formulated: A, C, E, G) 9 % w/w DPhB. B, D, F, H) 9 % w/w Texanol. Excitation was from the bottom through the cover slip.
In the Texanol formulated film a decrease in emission intensity was observed which resembled that of low $T_g$ latex films (Figure 4C, 4D). The emission maximum of dry film was red-shifted unexpectedly. In the fresh film it was at 453 nm, and in further 20 measurements it exhibited a hypsochromic shift of 6 nm. In the 1 week old dry film, however, it showed a bathochromic shift to 458 nm. The width was increased and skewness was decreased in fresh and 1 week old dry films.

The changes observed seemed smoother when the film was excited from bottom. The results obtained with 1-copolymerized low and high $T_g$ hydrophobic and hydrophilic latices showed a similar trend, i.e., emission intensity was decreased as film undergoes drying. There was no remarkable difference in the results obtained for low and high $T_g$ latices of hydrophobic and hydrophilic nature.

### 7.3.3 Change of Fluorescent Label

Considering the possibility that the decrease in fluorescence emission of the drying films was caused by photodegradation, or in any other way related with the photophysical characteristics of MFT, it was decided to change the label to an inert and photostable fluorophore. For this we selected Perylene Red (3), which is known to be a stable and very strongly fluorescent dye. The photostability of Perylene Red is even higher than that of Rhodamine dyes, which are well known for their photostability. For the experiments of film formation on the confocal microscope, we used Perylene Red and observed that the photostability of this dye was extraordinary (see Chapter 5). The laser power of excitation light on confocal microscope is many times higher than that in the steady-state experiments. So, the use of Perylene Red as label can rule out the speculation of photobleaching of 1-copolymerized latex film on the steady-state. Moreover the fluorescence of the model compound of 1 was found to be quenched in the presence of hydroxylic solvents. So aqueous phase of latex might affect the fluorescence of 1-copolymerized latex films.

A film of S/EHA60 latex was labeled with 10 ppm Perylene Red, and formulated with 9% w/w DPnB and studied with steady-state fluorescence spectroscopy. The results are shown in Figure 10. The method of excitation and collection of spectra was similar to that of the 1-copolymerized latex films. The excitation was at 560 nm and the detection wavelength was from 580 – 800 nm. Time for one measurement was 66 s. The instrument automatically took first 50 measurements during 55 min. After 1 week emission of dry film was measured and found to be very low.
Thus, the change of label did not solve the problem. The decrease in emission intensity was observed in low and high $T_g$ latices and results of 1-copolymerized or 3 physically added dye were similar. This shows that the role of photobleaching of the dye, during drying is unlikely to be important. Also, when the films were taken out of the sample chamber and measured again later, different spots in the sample were observed. If photobleaching would be strong, the later experiments should have shown fluorescence recovery. Another possibility of the decrease in fluorescence could be the inhomogeneous surface of the film. The film casting of the Texanol formulated latex provided more homogeneous and smooth films than a DPnB formulated latex.

### 7.3.4 Functionalization of Substrate

The latices used in the film drying experiments contained 60 % water, and in many cases it was observed that films of the dry latex did not have a smooth surface. Due to -OH groups present on the surface, the glass cover slip is hydrophilic, which may lead to dewetting phenomena. The contact angle between water and the surface is a measure of hydrophilicity of the surface. The contact angle of a clean glass cover slip was found to be $15^\circ \pm 2^\circ$. To increase the hydrophobicity of the glass cover slip it was functionalized with 3-aminopropyltriethoxysilane (APS) (Figure 11). Following the standard procedure of functionalization of glass surfaces a monolayer of APS was formed on the surface. The APS-functionalized substrate was then used to cast films for monitoring of film drying on the steady-state fluorescence.
Figure 11. Functionalization of glass cover slip by 3-aminopropyltriethoxysilane (APS) to increase the hydrophobicity of the surface.

Contact angle measurement revealed that functionalization of glass cover slips was successful and the water contact angle increased from $15 \pm 2^\circ$ to $71 \pm 2^\circ$ (Figure 12).

![Figure 12](image)

Figure 12. Contact angle measurement of water on APS-functionalized surface of substrate glass cover slip on three different positions in the film, A) before $\theta = 15 \pm 2^\circ$, B) after $\theta = 71 \pm 2^\circ$.

Latex film drying was monitored on the functionalized glass cover slip with steady-state fluorescence and results are presented in Figure 13. A film of 1-copolymerized S/EHA60 formulated with 9 % Texanol was cast on the APS functionalized glass cover slip of 60 x 24 mm having a thickness of 150 and 60 $\mu$m in wet and dry states, respectively. Fluorescence of fresh film was measured for 1 h and each measurement took 100s. Plots of emission maximum, intensity, width and skewness against time were similar to those observed in the previous experiments. Functionalization of substrate was not found to be helpful in solving the mystery of decrease of fluorescence of drying films. This shows that surface non-smoothness was probably not the reason for the decrease of fluorescence of drying films.
Figure 13. Fluorescence emission of drying film of S/EHA60 formulated with 9 % w/w Texanol: A) emission spectra, B) intensity versus time, C) emission maximum versus time, D) width versus time and E) skewness versus time. Substrate was APS-functionalized glass cover slip. $\lambda_{\text{exc}} = 380$ nm. Excitation was from the bottom through the cover slip.

### 7.3.5 Additives Free Latex Material

Film formation involves evaporation of water followed by particle deformation and coalescence. Interdiffusion of polymer chains is the final step for the completion of film formation. After water evaporation, all solid material remains on the substrate, which includes polymer and a number of additives present in the latex. In our emulsions additives include surfactant, iso ascorbic acid, tert-butyl hydroperoxide ($t$-BHPO) and
proxel ultra 10, which is an anti microbial agent that contains 9% 1,2-benzisothiazolin-3-one (BIT). During emulsion polymerization a post reaction treatment was carried out by adding a reducing agent (iso-ascorbic acid) together with t-BHPO. They work in combination to reduce the amount of free monomers. The level of free monomers in a latex emulsion must not exceed 500 ppm. In our low and high $T_g$ hydrophobic latex free monomers of styrene and 2-ethyl hexyl acrylate were in the range of 3 – 15 ppm and 6 – 200 ppm, respectively (see Chapter 2). In MMA/EA latex methyl methacrylate and ethyl acrylate were found in the range of 4 – 90 and 3 – 17 ppm, respectively. These numbers of free monomers in the latices were after the addition of iso-ascorbic acid and t-BHPO. To achieve the safe limit of free monomers (< 500 ppm) in latex without addition of iso-ascorbic acid and t-BHPO, an extra amount of initiator ammoniumpersulfate was added to reduce the amount of free monomers present at the end of emulsion polymerization. Absorption and emission of Proxel ultra 10 were measured (Figure 14). BIT exhibited emission from 350 – 600 nm, which might interfere with the apparent photophysical properties of the fluorescence dyes 1 and 2.

To monitor film drying with steady-state fluorescence the additives free MMA/EA latex was labeled with two different fluorescent dyes, namely Maleimidofluorotrope 2 and Perylene Red 3. Both were not chemically linked to the copolymer but just added to the latex after emulsion polymerization. These molecules being hydrophobic in nature it is expected that there is no chance for them to go into the aqueous phase, hence they were absorbed in the latex particles. Films were cast by the metallic bar to get wet and dry films of 150 and 60 $\mu$m thickness, respectively. Texanol and DPnB formulations were prepared (9 % w/w) of additives free MMA/EA60 latex. Fluorescence of drying films is presented in Figure 15. Surprisingly, the removal of additives produced very similar results as was observed with the additives containing latex.
A) Fluorescence emission of additives free MMA/EA60 latex labeled with 10 ppm MFT/n-butylamine adduct and formulated with 9 % w/w Texanol ($\lambda_{\text{exc}} = 380$ nm). B) Fluorescence of additives free MMA/EA60 latex labeled with 6 ppm Perylene Red and formulated with 9 % w/w DPnB ($\lambda_{\text{exc}} = 560$ nm). C) and D) intensity versus time plots of both films. Excitation was from the bottom through the cover slip.

Both films were measured for one hour and they exhibited the usual decrease in the fluorescence when films were drying. Apparently, the antimicrobial agent and the post-reaction treatment with iso-ascorbic acid were not the main causes.

### 7.3.5.1 Dialysis of Latex Material

Dialysis of additives free latex materials was carried out to remove water-soluble fluorescence quenchers as much as possible. The method of dialysis of latex is explained in section 7.8.2. After dialysis the fluorescence of neat additives free latex was measured. It was excited at two different wavelengths, i.e., 320 and 480 nm and spectra are shown in Figure 16.

After dialysis of additives free neat MMA/EA60 latex was formulated with co-solvent DPnB and labeled with fluorescent dyes 2 and 3 separately. These dyes were added to the latex by dissolving them in the co-solvent and formulation of latex was then carried out with this dye-loaded co-solvent. The concentration of 2 and 3 in the latex was 10 ppm.
Fluorescence of Drying Latex Films

Figure 16. Steady-state fluorescence emission of neat (nonlabeled) additives free MMA/EA60 latex: A) $\lambda_{\text{exc}} = 320\,\text{nm}$, B) $\lambda_{\text{exc}} = 480\,\text{nm}$.

Figure 17. Fluorescence emission of films of additives free MMA/EA60 latex after dialysis: A) formulated with 9\% DPNB labeled with 10 ppm compound 2. B) Formulated with 9\% DPNB labeled with 10 ppm compound 3. Formulation and labeling was carried out after dialysis of latex. Excitation was from the bottom through the cover slip.

Fluorescence of drying films of the additives free latex further cleaned by dialysis was measured on the usual glass cover slip of 60 × 24 mm. The thickness of wet and dry film was 150 and 60 µm, respectively. Results are shown in Figure 17. After dialysis there was still a decrease in the fluorescence during film drying, but this decrease was rather smooth.
as compared to previous experiments. Cleaning of the original latex that contained additives by dialysis was also performed, and results obtained were similar, i.e. a decrease of fluorescence was observed during film drying.

These experiments show that the decrease of fluorescence is slower when the latex has been cleaned. This suggests that water-soluble quenchers may play a crucial role in the decrease of fluorescence during drying.

7.4 Film Drying Studied with Time-Resolved Fluorescence

Film drying of latices was studied on the streak camera to investigate the fluorescence time profiles of the co-polymerized dye 1 in the latex. High $T_g$ hydrophobic and hydrophilic latices were employed in this experiment. Films of S/EHA and MMA/EA $T_g$ 60 °C formulated with 6 % DPnB were studied. In this experiment the original latex that contained additives was used. Films were cast by using the metallic bar producing 150 and 60 μm wet and dry film, respectively. After casting, the film was placed in the excitation beam path and the measurement was started. Drying of film was monitored for 30 min and 60 min in two different experiments. Decay profiles of 1-copolymerized S/EHA60 latex film were measured in 10 sequences on the streak camera. Time for each sequence was 3 min. During one sequence 18 scans were measured and exposure time was 10 s. Each sequence demonstrated an average lifetime of 18 measurements. During 30 min 10 sequences were measured in the wavelength range of 400 – 600 nm and during 60 min of film drying 20 sequences were measured. The excitation was done with a diode laser at 375 nm (pulse width ca. 50 ps). Biexponential models fitted the decay profiles well and yielded two lifetime components.

![Fluorescence decay](image)

In the first experiment 1-copolymerized S/EHA $T_g$ 60 °C formulated with 6 % w/w DPnB was employed. Table 1 presents the lifetime of the latex film during drying. A long time
component $\tau_1$ was ca. 9 ns with small contribution ($A_1 = 1 - 11 \%$) and a short time component $\tau_2$ was 2 ns with large contribution ($A_2 = 89 - 99 \%$). The decay profile was integrated over the wavelength range of the emission band. Figure 18 presents the decay profiles of 1-copolymerized S/EHA60 formulated with 6 % DPnB.

Table 1. Data obtained after fitting a biexponential model for decay of 1-copolymerized S/EHA60 6 % w/w DPnB formulated latex film on streak camera for 30 min (Figure 18). Fluorescence decay times were obtained by fitting the intensity integrated over the whole emission band.

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\(a)\) $\tau_{av} = (a_1 \tau_1^2 + a_2 \tau_2^2)/(a_1 \tau_1 + a_2 \tau_2)$, \(b)\) $f_1 = a_1 \tau_1/(a_1 \tau_1 + a_2 \tau_2)$ and \(c)\) $f_2 = a_2 \tau_2/(a_1 \tau_1 + a_2 \tau_2)$

Figure 19. Fluorescence decay of 1-copolymerized S/EHA60 6 % w/w DPnB formulated latex film on the streak camera for 60 min. $\lambda_{exc} = 375$ nm, $\lambda_{det} = 420 - 580$ nm.
Table 2. Data obtained after fitting biexponential model for decay of 1-copolymerized S/EHA60 6 % w/w DPnB formulated latex film on streak camera for 60 min (Figure 19). Fluorescence lifetime was obtained by fitting in the wavelength range of 420 – 580 nm.

<table>
<thead>
<tr>
<th>Sequence</th>
<th>Time constant 1</th>
<th>Time constant 2</th>
<th>( \tau_{av} )</th>
<th>( f_1 )</th>
<th>( f_2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( r_1 ) (ns)</td>
<td>( A_1 ) (%)</td>
<td>( r_2 ) (ns)</td>
<td>( A_2 ) (%)</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>9.9</td>
<td>8.2</td>
<td>2.0</td>
<td>91.8</td>
<td>4.4</td>
</tr>
<tr>
<td>2</td>
<td>10.3</td>
<td>12.4</td>
<td>2.3</td>
<td>87.6</td>
<td>5.4</td>
</tr>
<tr>
<td>3</td>
<td>9.5</td>
<td>9.5</td>
<td>1.9</td>
<td>90.5</td>
<td>4.5</td>
</tr>
<tr>
<td>4</td>
<td>10.1</td>
<td>8.1</td>
<td>1.9</td>
<td>91.9</td>
<td>4.5</td>
</tr>
<tr>
<td>5</td>
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<td>7.9</td>
<td>1.9</td>
<td>92.1</td>
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</tr>
<tr>
<td>6</td>
<td>9.1</td>
<td>3.4</td>
<td>1.5</td>
<td>96.6</td>
<td>2.8</td>
</tr>
<tr>
<td>7</td>
<td>10.3</td>
<td>20.7</td>
<td>2.8</td>
<td>79.3</td>
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<tr>
<td>8</td>
<td>9.8</td>
<td>5.2</td>
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<td>94.8</td>
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<tr>
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<td>11.8</td>
<td>2.2</td>
<td>88.2</td>
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</tr>
<tr>
<td>10</td>
<td>9.1</td>
<td>6.0</td>
<td>1.8</td>
<td>94.0</td>
<td>3.6</td>
</tr>
<tr>
<td>11</td>
<td>9.3</td>
<td>2.6</td>
<td>1.5</td>
<td>97.4</td>
<td>2.6</td>
</tr>
<tr>
<td>12</td>
<td>9.2</td>
<td>6.3</td>
<td>1.7</td>
<td>93.7</td>
<td>3.7</td>
</tr>
<tr>
<td>13</td>
<td>9.7</td>
<td>8.5</td>
<td>2.0</td>
<td>91.5</td>
<td>4.4</td>
</tr>
<tr>
<td>14</td>
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<td>1.8</td>
<td>94.6</td>
<td>3.6</td>
</tr>
<tr>
<td>15</td>
<td>9.3</td>
<td>0.6</td>
<td>1.2</td>
<td>99.4</td>
<td>1.6</td>
</tr>
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<td>10.9</td>
<td>2.6</td>
<td>89.1</td>
<td>2.5</td>
</tr>
<tr>
<td>17</td>
<td>8.6</td>
<td>1.4</td>
<td>1.3</td>
<td>98.6</td>
<td>5.4</td>
</tr>
<tr>
<td>18</td>
<td>8.7</td>
<td>1.8</td>
<td>1.3</td>
<td>98.2</td>
<td>2.1</td>
</tr>
<tr>
<td>19</td>
<td>10.1</td>
<td>10.6</td>
<td>2.3</td>
<td>89.4</td>
<td>5.0</td>
</tr>
<tr>
<td>20</td>
<td>9.2</td>
<td>5.2</td>
<td>1.8</td>
<td>94.8</td>
<td>3.4</td>
</tr>
</tbody>
</table>

\[ a_{mv} = \frac{(a_1 r_1^2 + a_2 r_2^2)}{(a_1 r_1 + a_2 r_2)}, \quad b_{f_1} = \frac{a_1 r_1}{(a_1 r_1 + a_2 r_2)} \quad \text{and} \quad c_{f_2} = \frac{a_2 r_2}{(a_1 r_1 + a_2 r_2)} \]

Another film of the same latex, i.e., 1-copolymerized S/EHA60 formulated with 6 % DPnB was studied for 60 min and the decay profiles of the drying film were measured in 20 sequences. Data obtained are shown in Table 2. The fluorescence decay of S/EHA60 latex film studied for 60 min also showed two lifetime components. An average lifetime was calculated and found to vary from 2 to 5 ns.

The polymer phase in which the probe is covalently integrated is not microscopically homogeneous so it is not surprising to see non-exponential decay.
Compound 2, which is a model compound of 1, exhibited monoexponential decay in various organic solvents and biexponential decay in co-solvents. The lifetime was higher (ca. 14 ns) in nonpolar solvents and lower (0.17 ns) in polar acetonitrile. In DPnB and Texanol decay of 2 was biexponential: \( \tau_1 = 2.2 \) and 2.5 and \( \tau_1 = 0.5 \) and 0.4 ns, respectively. The decay profile of 1-copolymerized latices were measured in the bulk and the results are shown in Table 3.

Table 3. Fluorescence decay of 1-copolymerized latices measured in bulk (cuvet) on the streak camera. A biexponential model was applied to fit the intensity profile integrated over the emission band.

<table>
<thead>
<tr>
<th>S/EHA Latex / Co-solvent (ppm)</th>
<th>Copolymerized 1 ( \lambda_{\text{max}} ) (nm)</th>
<th>( \tau_1 ) (ns)</th>
<th>( A_1 )</th>
<th>( \tau_2 ) (ns)</th>
<th>( A_2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( T_g 20 , ^\circ \text{C} / 3 % \text{DPnB} )</td>
<td>6</td>
<td>454</td>
<td>10</td>
<td>0.8</td>
<td>2.5</td>
</tr>
<tr>
<td>( T_g 20 , ^\circ \text{C} / 9 % \text{DPnB} )</td>
<td>6</td>
<td>470</td>
<td>11</td>
<td>0.8</td>
<td>2.7</td>
</tr>
<tr>
<td>( T_g 20 , ^\circ \text{C} / 3 % \text{Texanol} )</td>
<td>6</td>
<td>457</td>
<td>11</td>
<td>0.8</td>
<td>3.2</td>
</tr>
<tr>
<td>( T_g 20 , ^\circ \text{C} / 9 % \text{Texanol} )</td>
<td>6</td>
<td>471</td>
<td>11</td>
<td>0.7</td>
<td>2.9</td>
</tr>
<tr>
<td>( T_g 20 , ^\circ \text{C} / 3 % \text{DPnB} )</td>
<td>18</td>
<td>454</td>
<td>11</td>
<td>0.8</td>
<td>4.5</td>
</tr>
<tr>
<td>( T_g 60 , ^\circ \text{C} / 3 % \text{DPnB} )</td>
<td>6</td>
<td>453</td>
<td>11</td>
<td>0.7</td>
<td>3.2</td>
</tr>
<tr>
<td>( T_g 60 , ^\circ \text{C} / 9 % \text{DPnB} )</td>
<td>6</td>
<td>463</td>
<td>11</td>
<td>0.7</td>
<td>2.9</td>
</tr>
</tbody>
</table>

The decay times found in the wet latex are similar to those found in the drying films, but in the wet state the long lifetime component is more important.

Results of film drying on the streak camera showed that 1-copolymerized latex exhibits two lifetimes: the long lifetime component was found to have a small contribution and the short lifetime component was prominent. Most importantly, the decrease of the average lifetime during drying does not match the decrease in the fluorescence intensities. There is no indication of a change in the dynamics of fluorescence quenching during drying. Thus, it seems that an increasing fraction of the probe molecules becomes non-fluorescent during drying.

7.5 Discussion

Drying of low and high \( T_g \) hydrophobic and hydrophilic latices was studied by means of steady-state and time-resolved fluorescence spectroscopy.

The co-polymerized probe MFT (1) and its model compound (2) are solvatochromic fluorophores, which are very sensitive to the polarity and mobility of the medium. These probes are sensitive to viscosity because the stabilization of the excited state dipole requires medium reorganization.\(^{14-16}\) Therefore, the increase in the rigidity of the medium results in the blue shift of 1 and 2 when films are dry.
The fluorescence intensity of a dry latex film is expected to remain essentially the same as that observed in the fresh film. Since the total amount of fluorescent label in the observed area remains the same when the thickness of the film decreases, only intrinsic changes of the fluorescence of the probes should be observed. The strong decrease in fluorescence intensity of labeled latex films during drying, as observed by steady-state and time-resolved emission, was therefore unexpected.

In the steady-state fluorescence measurements a remarkable feature was the rise of emission intensity at ca. 20 min of drying time for the low \( T_g \) latices (Figure 4A, 4B), which lasted for a short time, but then was followed by a gradual decrease. In high \( T_g \) latices this feature was pronounced for the Texanol (Figure 9B) formulated latex but in the case of DPnB it appeared as a small shoulder in the intensity versus time plot at ca. 20 min (Figure 9A). From the film drying experiments performed on the confocal microscope in one dimensional mode (1D) and those of gravimetric analysis we observed that > 90 % water has evaporated from the film at 15 – 20 min drying time (see section 5.3 Chapter 5). The rise and fall of emission intensity in film drying around 20 min is probably related to the end of the water evaporation stage.

We performed several experiments to find the cause of the decrease in emission intensity as a function of time during film drying. To avoid oxygen, a potential quencher of fluorescence, an experiment was performed in a nitrogen atmosphere, but it produced similar results. A more photostable and inert fluorescent label was tried to avoid all effects that might be caused by the photobleaching or to the photophysical peculiarities of copolymerized 1, such as quenching by water. For this purpose, Perylene Red (3) was used, which provided nice results when used to observe individual latex particles on the confocal microscope (Chapter 5) due to its excellent photostability. Unfortunately labeling with 3 did not help and fluorescence intensity still decreased in drying films. The same observation was made in the 1D scanning experiments in Chapter 5 in which the thickness of the film during water evaporation was monitored.

The drying of films from latex that contain surfactant is not homogeneous: the drying proceeds by a propagating front. The drying process is faster on the edges than in the middle of the film. We observed that drying films have transparent and turbid regions due to the propagating front and inhomogeneous drying. We considered the possibility that the change of surface texture of the film during drying might change the amount of scattered light and thereby affect the observed fluorescence intensity. This should be much less of a problem when the film is observed from below, through the cover slide. When observed in this way, the fluorescence seemed to change in a smoother way than when observed from above, but the overall intensity decrease was still dramatic.
Another possibility is that partial dewetting occurs during film drying, which may lead to a smaller amount of fluorescent label in the observed area. In an attempt to change the wetting properties of the substrate, functionalization of the glass cover slip with 3-aminopropyltriethoxysilane (APS) was performed. The water contact angle of the functionalized glass cover slip was > 70° showing that its surface was less hydrophilic. However, the results of films drying obtained using the functionalized substrate were not very different from those in which the substrate was not functionalized.

When a film is drying, water evaporates, leaving the solid content on the substrate. In our latices water to solid content ratio was 60 : 40 %. In addition to polymer, the solid content consisted of surfactant and additives. The addition of additives is crucial to carry out post-reaction to suppress the residual monomer content, and to produce a more stable emulsion with longer shelf life. Additives include iso-ascorbic acid, t-BHPO and Proxel ultra 10 which is a formulation containing 9 % BIT against microbial attack. The ions from initiator also remain in the solid content of the film. It is not well known what happens with the additives during film formation. After application of wet latex on the substrate, water evaporates and additives might come in close contact with the dye, which might lead to quenching of its fluorescence. In particular, the fluorescence of dyes 1, 2, and 3, can relatively easily be quenched by electron transfer from an electron donor. A batch of additives free hydrophilic latex was prepared and labeled (non-covalently) with MFT/n-butylamine adduct (2) and Perylene Red (3) in separate batches. During film drying of such a latex, however, we still observed decrease in the fluorescence. To further clean the latex, dialysis was carried out to get rid of any water-soluble quenchers that might be present. The film drying experiment of “clean” additives free latex still showed a decrease in intensity however it was markedly slower than in the case of the standard latices, showing that water soluble quenchers might be one of the possible reason of decrease in intensity.

Time-resolved experiments showed that the average lifetime of the 1-copolymerized hydrophobic latex films is shorter than in the wet latex, but does not change much during drying. This means that if quenching is the main cause of intensity decrease, it is of a static nature, not dynamical quenching.

The surfactant is one aspect that must not be neglected. It hinders and disrupts the particle ordering during film drying and there is a possibility that the surfactant causes the quenching of fluorescence during film drying. More experiments need to be performed to further investigate this matter. One option to be explored is film drying with surfactant free latex films.
Chapter 7

7.6 Conclusion

Film drying of low and high $T_g$ hydrophobic and hydrophilic acrylic latices was studied with steady-state and time-resolved fluorescence spectroscopy. A solvatochromic probe 1 and its derivative 2 were polarity and mobility sensitive probes. Perylene Red 3 was a position sensitive probe. A strong decrease in the fluorescence intensity of drying films was observed. Low and high $T_g$ latices showed similar results and the reason for this decrease is not yet fully understood. Various experiments showed that the uneven texture during drying may play a role, but water-soluble additives or contaminants present in the latex appeared to be the main reason for the decrease in emission. The surfactant present in the latex remains in the dry film, even after dialysis, which might be a possible cause of fluorescence quenching.

7.7 Experimental Details

7.7.1 Preparation of Additives Free Latex

Hydrophilic methyl methacrylate-co-ethyl acrylate co-polymers (MMA/EA) high $T_g$ additives free latex was prepared via emulsion polymerization. The $T_g$ of final latex was designed to be 60 °C according to the Fox equation (Chapter 2).

The ratio of monomers was 2.75 : 1 and water to solid ratio of the final latex was 60 : 40 %. This latex was prepared without labeling of fluorescent dye. Table 4 presents the recipe of emulsion polymerization.

Table 4. Emulsion polymerization recipe for the preparation of additives free nonlabeled high $T_g$ methyl methacrylate-co-ethyl acrylate (MMA/EA).

<table>
<thead>
<tr>
<th>Component</th>
<th>Weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>885</td>
</tr>
<tr>
<td>Sodium laurylsulfate (SLS)</td>
<td>19.7</td>
</tr>
<tr>
<td>Ammonium persulfate (APS)</td>
<td>118 + 1.2</td>
</tr>
<tr>
<td>Acrylic acid</td>
<td>11.8</td>
</tr>
<tr>
<td>Methyl methacrylate (MMA)</td>
<td>425.1</td>
</tr>
<tr>
<td>Ethyl acrylate (EA)</td>
<td>154.2</td>
</tr>
</tbody>
</table>

Emulsion polymerization was carried out in a 2 L reactor equipped with mechanical stirrer with variable speed (100-1000 rpm) and reflux condenser, all under $N_2$ atmosphere. The reactor was charged with water and 1.3 % sodium lauryl sulfate (SLS). Ammoniumpersulfate (APS) 0.12 % was used as initiator in the emulsion polymerization. 5% Of the monomers was introduced at once to the solution of water and SLS in the
reactor at 50 °C and 30 % of initiator solution was added to the reactor at once at 70 °C. Then the temperature of the reactor was increased up to 85 °C and the remaining monomer and initiator feeds were added to the reactor over a period of 90 min at this temperature. After finishing monomer and initiator feeds polymerization was allowed to continue for 30 min. This latex was free from additives (iso-ascorbic acid, tert-butyl hydroxy peroxide (t-BHPO) and Proxel ULTRA10) therefore post reaction was performed by dissolving 1.2 g ammoniumpersulfate (APS) in 30 ml of demi water and added to the reactor. After 30 min the reaction mixture was cooled down from 85 °C to room temperature. Finally the latex was filtered through a 75 μm filter cloth and stored in clean bottles.

7.7.2 Dialysis of Latex

Dialysis of additives free latex was carried out to clean it from water-soluble fluorescence quenchers. Dialysis tubes Platgem 26 mm Visking, (TWT-400-050S, 30 m) were filled with 10 ml of additives free MMA/EA60 latex and left for 36 hours in a cylinder filled with milli-Q water, equipped with a magnet bar for slow stirring. Milli-Q water was refreshed twice during this experiment to maximize the removal water-soluble material.

7.7.3 Steady-State Fluorescence

The fluorescence emission spectra of labeled latex films were recorded on glass cover slip of 60 × 24 mm on a Spex Fluorolog 3 spectrofluorimeter equipped with two double monochromators. The excitation and emission wavelengths of 1-copolymerized and 2-labeled latices were 380 nm and 400 – 600 nm, respectively. For 3-labeled latices excitation was at 560 nm and detection at 580 – 800 nm. The detector was a Peltier-cooled R636-10 (Hamamatsu) photomultiplier tube. Spectra were corrected for the wavelength dependence of the detection system. Measurements carried out in steady-state fluorescence were at room temperature (20 °C). The spectral shape was characterized by fitting the spectra $I(\lambda)$ with a skewed Gaussian function (equation 1). For more details see Chapter 2.

7.7.4 Streak Camera

Time-resolved fluorescence of 1-copolymerized latex was studied with a Hamamatsu streak camera system consisting of a Chromex IS250 spectrograph, a M5677 slow-speed sweep unit, a C4792 trigger unit, a C5680 blanking unit and C4742-95 digital CCD camera. Excitation was from a diode laser (50 ps FWHM, 375 nm). Excitation and emission light were guided via optical fibers in a front-face geometry.
7.8 References


