PII: S0032-3861(96)01063-4

Polymer Vol. 38 No. 12, pp. 2899–2906, 1997

0032-3861/97/$17.00 + 0.00

Fluorescence lifetime studies of labelled polystyrene latices

J. W. Hofstraat*
Akzo Nobel Central Research Laboratories, PO Box 9300, NL-6800 SB Arnhem, The Netherlands

and H. J. Verhey and J. W. Verhoeven
Department of Physical Organic Chemistry, University of Amsterdam, Amsterdam, The Netherlands

and M. U. Kumke, G. Li, S. L. Hemmingsen and L. B. McGown
Department of Chemistry, Duke University, Durham, NC, USA
(Received 24 November 1995; revised 15 July 1996)

A charge-transfer fluorescence molecule has been applied to study the structure of polystyrene latices and polystyrene–glycidylmethacrylate core–shell latices. Information on the composition of the latex could be obtained on the basis of the combined application of steady state and time-resolved fluorescence measurements. Time-resolved measurements were done using a frequency-domain approach; the time-resolved data were interpreted using the maximum entropy method of analysis. © 1997 Elsevier Science Ltd.

(Keywords: fluorescence; time-resolved fluorescence; charge transfer)

INTRODUCTION

Latices find widespread application in areas like diagnostics and advanced, water-based, coatings. In particular polystyrene-based latices are used, since they can be prepared under very controlled circumstances by emulsion polymerization. The final molecular weight of the polystyrene particles and hence the dimensions of the particles can therefore be accurately determined. Polystyrene particles are thus also very suitable for reference purposes, for instance for quality control of particle size instrumentation or flow cytometers.

Subjects which are of interest for the application of latices are: (1) the conditions under which optimum loading with a label (either a strongly coloured or fluorescent material) is achieved; (2) the possibilities of modifying the surface of the particles so that specific interactions with proteins (in diagnostics) or with crosslinkers (in coatings) become possible. Both these subjects have been examined previously for a polystyrene latex and a polystyrene-glycidylmethacrylate core–shell latex by steady state fluorescence techniques[1]. In the previous work the luminescent probe 1-phenyl-4-[(cyano-1-naphthyl)methylene]piperidine, hereinafter referred to as Fluoroprobe, was used. The Fluoroprobe molecule has a highly dipolar excited state, which is populated by excitation in the ultraviolet (u.v.) range by intramolecular electron transfer. The strong dipolar character of the excited state induces a strong response of the fluorescence spectrum to changes in the polarity and the viscosity of the medium: in polar and low viscosity solvents the excited state is stabilized to a large extent, which leads to a red shifted fluorescence spectrum; in apolar and highly viscous solvents the fluorescence is located in the blue[2].

On the basis of the changes of the fluorescence spectrum of Fluoroprobe in polystyrene particles the swelling process induced by the addition of low mole-cular weight compounds to the latex could be monitored. Also, the formation of polystyrene–glycidylmethacrylate core–shell particles, produced by polymerization of the acrylate after addition to the latex, could be followed with Fluoroprobe[1]. In both investigations steady state fluorescence detection was employed. Since the fluorescence emission spectra are rather broad, as is usual for charge-transfer fluorescence, the application of steady state methods is limited to rather qualitative observations. In particular, it is difficult to distinguish several coinciding species. For evaluation of heterogeneous systems, such as polymeric materials, where many species with different microenvironments are expected, the steady state method has its limitations. The use of the fluorescence lifetime as an additional observable may help to get a more accurate picture of such complicated structures.

The purpose of this study is to show the power of combined steady state and time-resolved measurements of charge-transfer probe fluorescence in latex systems, as an illustration of the potential of this technique for polymer analysis.

Time-resolved fluorescence measurements

The time dependence of the distribution of excited

* To whom correspondence should be addressed

states created by absorption of light is an observable which opens a new dimension in the interpretation of fluorescence spectra and, based on this additional information, provides insight into fast polymer dynamics[3,4]. Basically two types of applications of fluorescence lifetime spectroscopy can be envisaged. In one, the time dimension of the fluorescence signals is used as extra discriminatory tool (in addition to the excitation and emission wavelengths). For this purpose it is generally sufficient to measure the decay with time of the fluorescence intensity; the time-dependent intensity is subsequently fitted to a model to determine the lifetime, or possibly lifetimes, of the fluorescent species:

$$I(t) = \sum_i a_i \exp(-t/\tau_i)$$

in which a_i gives the relative weight of the component with lifetime τ_i.

In the second application, fluorescence lifetime data are used to interpret interactions (e.g. energy transfer) or dynamics of fluorophores. Of course, direct lifetime analysis, using the approach described above, can be used for this application as well. A more powerful approach, however, is to measure the dynamic anisotropy of the fluorescence. In this kind of measurement a photoselected, anisotropic population of excited molecules is created by means of a polarized light source. The loss of anisotropy with time is subsequently measured; the time-resolved anisotropy is both influenced by molecular motions (and hence related to parameters like viscosity of the solvent and size of the fluorescent species) and by energy transfer mechanisms, which result in depolarization.

The time-resolved anisotropy function $r(t)$ is determined via measurement of the two polarized emission decays, $I_p(t)$ and $I_s(t)$, where I_p and I_s are the fluorescence intensities parallel and perpendicular to the polarization state of the excitation beam:

$$r(t) = \frac{I_p(t) - I_s(t)}{I_p(t) + 2I_s(t)}$$

Measurement of time-resolved fluorescence spectra requires the application of dedicated methods, since time-resolved data starting from the low ps-range are required. Two main methods are employed for time-resolved fluorescence measurements[5]. One is the time-correlated single photon counting technique, in which the time-resolved response is evaluated of a system brought into the excited state by means of a short (ps) laser pulse. The second approach is to excite the sample with an excitation beam which is modulated in intensity by, for instance, an electro-optic shutter. The light intensity can thus be modulated up to high frequencies; the fluorescence is measured by application of phase-sensitive detection[6]. The demodulation and the phase shift between the excitation beam and the fluorescence signals can be measured as a function of the modulation frequency. The demodulation and phase shift variation as a function of frequency again can be fitted with a model to derive the fluorescence lifetimes.

Time-resolved fluorescence techniques offer an interesting opportunity to get an insight into the structure of heterogeneous systems. Fluorescence lifetime, in addition to spectral data, provides information on sample composition. The use of standard data analysis techniques to resolve complicated decays of heterogeneous samples is often inadequate. This has prompted the development of new strategies for data analysis to improve the accuracy and reliability of the lifetime information extracted from the data. An analysis method with promise for time- and frequency-domain lifetime data is the maximum entropy method (MEM)[7-11]. Standard lifetime data analysis algorithms utilizing nonlinear least squares (NLLS) require the prior selection of a model comprised of some small number (typically less than five) of discrete and/or distributed exponential decays. The algorithm is constrained to fit the number and type of decays in this a priori model. One difficulty in this approach is that continuously distributed decays are modelled by simple functions (e.g., Gauss or Lorentzian), that may oversimplify the true distributions and often have no physical foundation. Such oversimplifications are further complicated by ambiguities in the results of the χ^2 goodness-of-fit criterion that is used to select the best model. In contrast to the standard NLLS analysis, the MEM approach recovers a lifetime distribution that is unbiased by any a priori model selection: the shape of the distribution does not need to follow a predetermined equation, and MEM can therefore be considered to be 'self-modelling'. The essential difference between MEM and other approaches which are self-modelling, such as the exponential series method (ESM[12]), is that MEM utilizes an additional constraint, the so-called Shannon–Jaynes entropy constraint, which requires that a maximum value should be found for the statistical entropy function:

$$S = -\sum_i p_i \log p_i$$

where p_i is the fractional intensity for every lifetime component considered in the fitting process. The crucial result of the application of the entropy constraint is that it selects a unique distribution among those that meet the χ^2 criterion.

EXPERIMENTAL

Materials

Styrene from Baker was purified over Al_2O_3 (Janssen) white quartz sand (Aldrich) to remove the stabilizer. Potassium persulfate (Baker) initiator and sodium hydrogen carbonate (Baker) buffer were used as received. Aerosol MA-80 surfactant from American Cyanamid was applied as 80% solution in isopropanol. Ultra pure water was obtained from a Millipore Milli-Q plus system. Glycidylmethacrylate (from Aldrich) was used as received. Fluoroprobe was synthesized as reported in ref. 2.

Latex preparation

The preparation of the styrene latex and the preparation of the glycidylmethacrylate–styrene core–shell latex were done by emulsion polymerization. Details of the preparation procedures were reported in ref. 1. The Fluoroprobe label was introduced into the particles by means of a swelling procedure as suggested by Ugelstad[13].

Polymerization of glycidylmethacrylate

Glycidylmethacrylate was polymerized in bulk in the presence of 1.5 wt% acrylamide-functionalized Fluoroprobe,

by application of benzoylperoxide as initiator at a temperature of 70°C. The polymerization was followed via measurement of the fluorescence emission spectrum of the Fluoroprobe label. The reaction was completed after 300 min of reaction: the wavelength of the fluorescence emission did not change after this reaction time.

Measurements

Steady-state fluorescence data were acquired with a Spex Fluorolog 2 spectrofluorometer in right-angle geometry on latices which had been diluted 1/2000 with a 1 wt% solution of Aerosol MA-80 in water. The spectra were corrected for detector response. Excitation was done at 310 nm.

Time-resolved measurements on 1/2000 diluted latices in aqueous dispersion were performed with a 4850 multi-harmonic Fourier-transform (MHF) phase-modulation fluorometer from SLM Instruments, using a cross-correlation detection scheme[14]. A HeCd laser (Liconix) was used for excitation at 325 nm. Various Oriel band-pass and cut-off filters were used to select the emission wavelength. The dynamic measurements were made with a base frequency of 5.0 MHz. Data were collected at 40 frequencies in the range of 5–200 MHz. The base cross-correlation frequency for detection was 4.167 Hz. The fluorescence phase angle differences (PADs) and modulation amplitude ratios (MARs) were calculated as the averages of 15 replicate measurements, where each measurement was the average of 100 samples taken over a period of 24 s.

Two types of measurement were performed. Firstly, fluorescence lifetime measurements were made in a 90° configuration. The emission polarizer was set to the magic angle to eliminate photoselection effects. The time-response of a scattering solution of glycogen in water, intensity matched to the sample, was used as lifetime reference. Secondly, dynamic fluorescence anisotropy (DFA) measurements were done in the T-format. In this experiment the excitation polarizer is set to pass vertically polarized light, and one of the two emission polarizers is alternated between horizontal and vertical polarization, while the other is set at horizontal to serve as a reference.

The temperature of the sample compartment was kept fixed at 20.0 ± 0.1°C by a circulating water bath.

Data analysis and computation

Analysis of the lifetime and DFA data was done by NLLS analysis software from Globals Unlimited (Version 3). Lifetime data were also analysed by MEM software from Maximum Entropy Data Consultants Ltd. Goodness of fit for NLLS analysis of fluorescence lifetime and rotational correlation time data was evaluated for various models (one-, two- and three-component models) by examination of the reduced χ^2 values and of the randomness of the distribution of residuals across the modulation frequency range.

RESULTS AND DISCUSSION

Steady state fluorescence

The steady state fluorescence spectra shown in *Figure 1*, for Fluoroprobe in a homogeneous solvent, toluene, and for Fluoroprobe introduced into a polystyrene latex and into a polystyrene–glycidylmethacrylate (GMA) core–shell latex demonstrate the strong dependence of the photophysical behaviour of this probe molecule on the polarity and viscosity of the solvent. The spectra were obtained via excitation at 310 nm. The emission maximum of Fluoroprobe in toluene, a rather apolar solvent, is at 475 nm. Fluoroprobe in polystyrene, essentially a matrix with the same polarity as toluene, has a fluorescence emission maximum at 445 nm. The blue shift can be attributed to the higher viscosity of the polymer matrix. When Fluoroprobe is introduced into a core–shell latex a more complicated spectrum is obtained, in which three emission maxima are observed, at 380 nm, 410 nm and 450 nm. The 380 nm fluorescence is due to a decomposition product of Fluoroprobe. This component is especially observed when the Fluoroprobe molecule has been exposed to radical curing conditions. The luminescence wavelength is shorter than observed for Fluoroprobe in any solvent. In view of the photophysical properties of the decomposition product, it might be formed via degradation of the aniline donor, leaving a vinylcyanonaphthalene fluorophore. The other two maxima can be attributed to Fluoroprobe in two, different environments: a polystyrene-like environment, which gives rise to the 450 nm band and a much more rigid or apolar environment. To be able to interpret the origin of the 410 nm band, which one would assume to arise from Fluoroprobe in a poly-GMA environment, the bulk polymerization of GMA has been studied in the presence of Fluoroprobe. The polymerization was carried out at 70°C using the thermal initiator benzoylperoxide. The fluorescence spectra of Fluoroprobe recorded during the reaction are shown in *Figure 2*. The strong dependence of the emission spectrum of Fluoroprobe on viscosity is even more obvious from the impressive blue shift of the emission maximum during the reaction: at the beginning of the reaction the emission maximum of Fluoroprobe in the liquid GMA (which is rather polar) is about 655 nm. At the end of the reaction, the solid poly-GMA, which is still a rather polar matrix, shows an emission maximum of Fluoroprobe of 514 nm. The blue shift is mainly attributable to the large increase in viscosity of the solvent. In view of the observations for the behaviour of Fluoroprobe in poly-GMA, it is most likely that the 410 nm emission stems from Fluoroprobe in a very rigid environment, for instance formed by an interpenetrating network of polystyrene and GMA[1]. On the basis of steady state fluorescence spectra it is not possible to make any firmer assignments. Additional information is obtained when the fluorescence lifetime behaviour is considered.

Time-resolved measurements: fluorescence lifetimes

Fluorescence lifetimes recovered from NLLS analysis approach for the three systems described above are given in *Table 1*. The difference between the liquid (toluene) solution and the two polymer samples is striking. The liquid solution has only one fluorescence lifetime, which is strongly dependent on the presence of oxygen in the solution. In a solution saturated with nitrogen the fluorescence lifetime is more than 50% longer than in the oxygenated solution, where the excited state of Fluoroprobe is quenched by the interaction with oxygen. The relative lifetimes are in good accordance with the Stern–Volmer equation, when the fluorescence lifetime and the diffusion rate of oxygen in toluene are taken into account.

In the two polymer matrices the fluorescence lifetime analysis yields a much more complicated picture. The χ^2

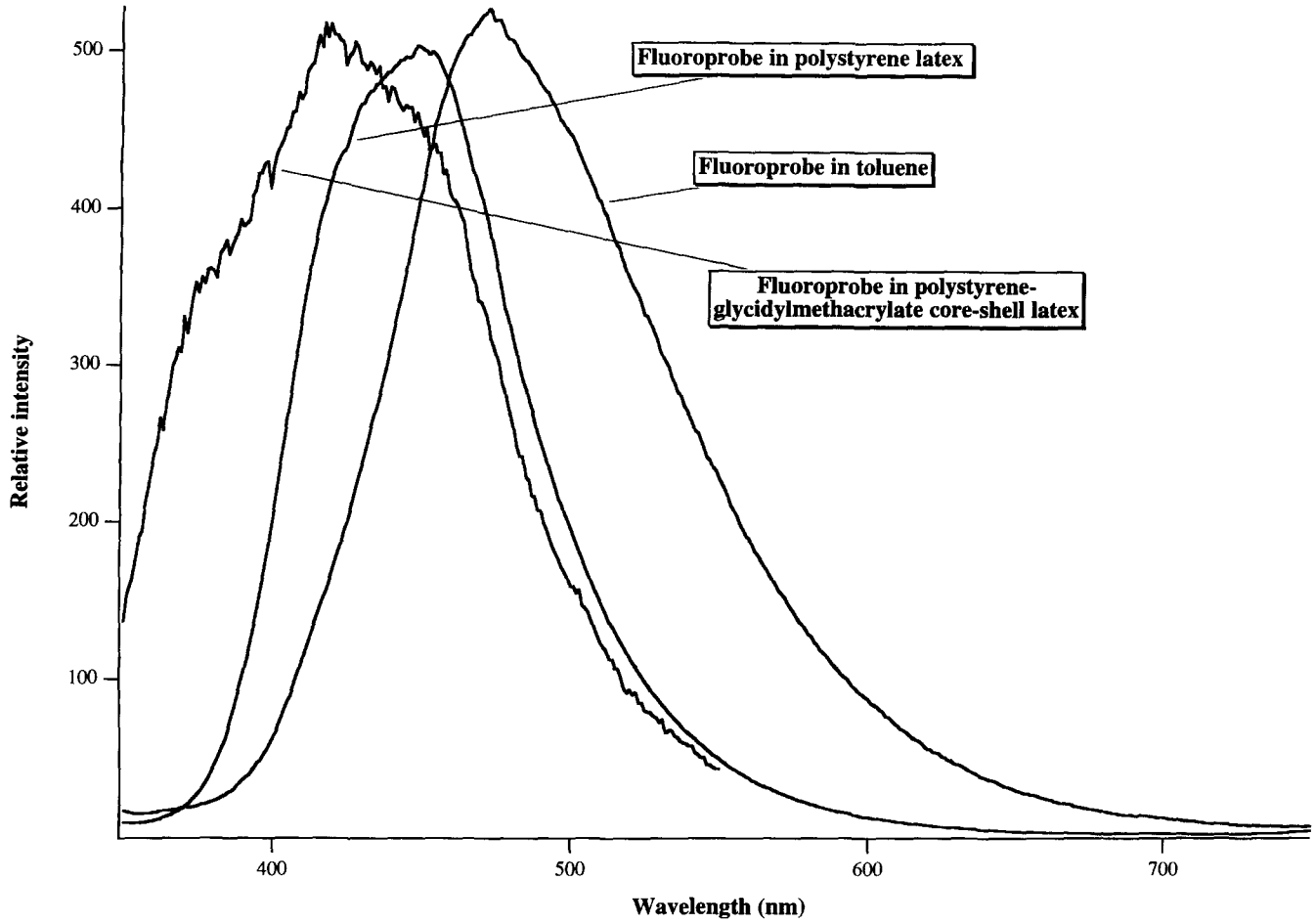

Figure 1 Steady-state fluorescence spectra of Fluoroprobe in toluene, polystyrene latex and in polystyrene–GMA core–shell latex. Excitation was at 310 nm. Spectra do not reflect actual fluorescence response, but have been scaled to maximum intensity

value obtained for single exponential fits is high, 10^2–10^3, for all measurements. In general at least two, and usually even three, lifetime components are recovered, which are strongly wavelength dependent. This observation suggests that the polymer matrix is very heterogeneous. The tendency of the data is that the longer the emission wavelength used for detection, the longer the fluorescence lifetimes which are recovered. This can be understood by invoking the previously discussed picture of charge-transfer fluorescence, in which the red shifted fluorescence is due to more relaxed fluorophores. The difference between the polystyrene and the polystyrene/GMA latices is not significant, according to the fluorescence lifetime data. Only by using discrete lifetime values via NLLS analysis could reasonably good fits be obtained. Efforts to model the fluorescence lifetime distribution by making use of Gaussian lifetime distributions in the NLLS program led to erroneous results: in many cases even 'negative' lifetimes were obtained.

Subsequently, the same data were subjected to MEM analysis. The results are given in *Table 2*; the recovered lifetime distributions are depicted in *Figure 3*. For reference purposes the distribution recovered for Fluoroprobe in toluene solution is also shown. The fluorescence lifetime recovered for the solutions is the same as derived via the NLLS analysis (see *Table 1*). The lifetime distribution is extremely narrow ('single exponential') for the toluene solutions, which is consistent with the NLLS analysis. The polymer latices give much broader lifetime distributions. The heterogeneous structure of the latices, which was suggested by the NLLS analysis, is therefore confirmed by the MEM data. The results of the MEM analysis also suggest a difference between the polystyrene and the polystyrene/GMA latices: the lifetime distribution in the polystyrene latex is clearly different from that observed for the core–shell latex (see *Figure 3* and *Table 2*). The MEM method of analysis appears to provide clear fluorescence lifetime results, which do not require supervised or subjective calculations, and provides an insight into the distribution of lifetimes in heterogeneous systems.

A further question is whether we are able to shed some light onto the actual structure of the core–shell latex. To be able to interpret the data several additional experiments were executed: fluorescence lifetimes were measured for Fluoroprobe in polystyrene film and in a pure GMA polymer sample. The results are given in *Table 3*. The fluorescence lifetime analysis of Fluoroprobe in polystyrene film yields results which are similar to those obtained in solution: a relatively narrow, heterogeneous distribution with three relevant lifetimes. The lifetime distribution of the polystyrene film sample is shifted to somewhat longer lifetime values. A difference between the two types of samples is, of course, that in the latex the polymer particles are in equilibrium with the surrounding solvent, water. Swelling of the polymer particles by solvent or interaction of the fluorophores with water, e.g. the outer parts of the particles, will lead to significant decreases of the fluorescence lifetimes. The lifetime results suggest that also in the dispersion the

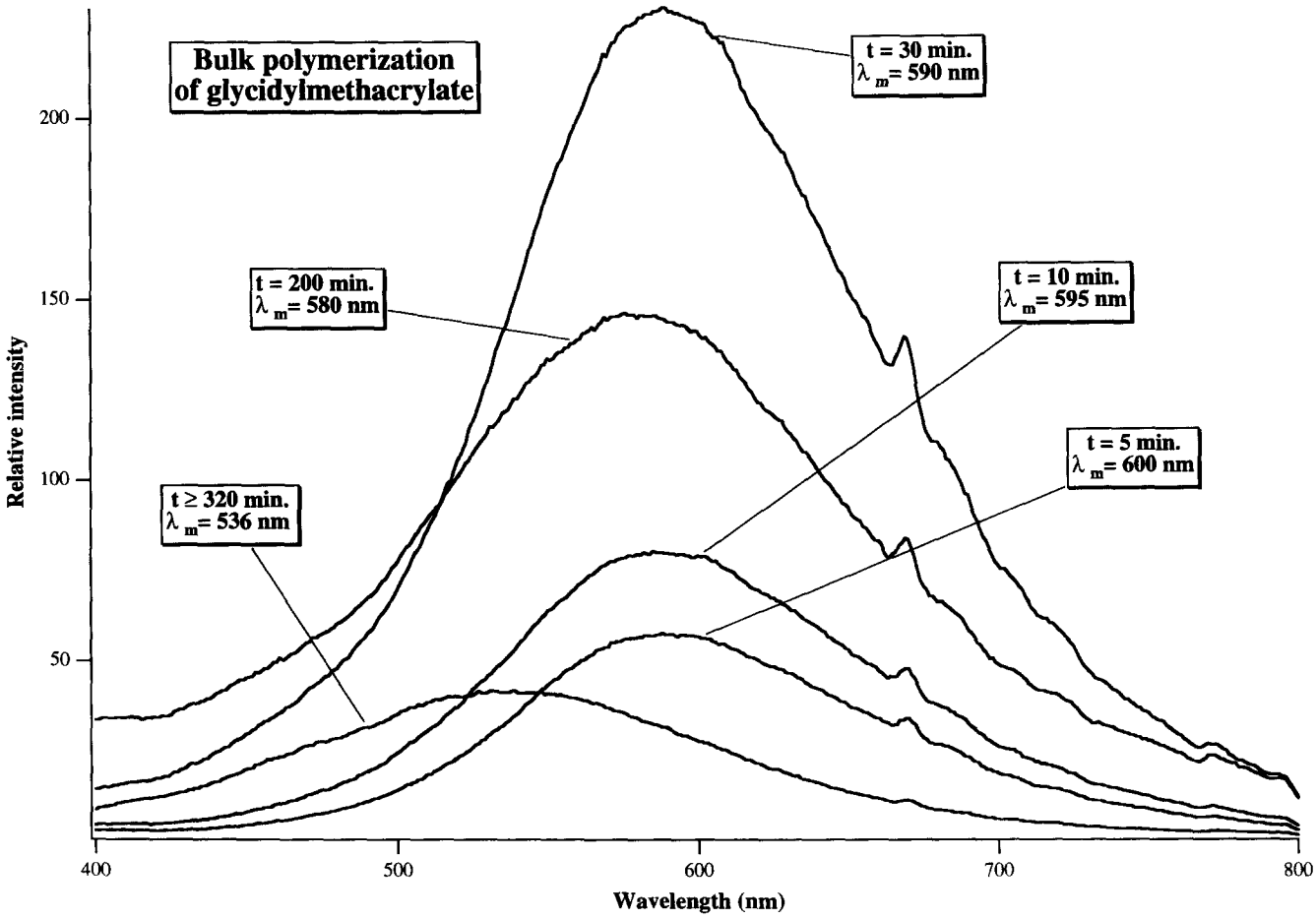

Figure 2 Steady-state fluorescence spectra of Fluoroprobe in polyglycidylmethacrylate; recorded during the polymerization

Fluoroprobe is *inside* the latex particles. This is consistent with the spectra, which are very similar for both types of matrices, cf. *Figure 1*.

Another system which was studied is that of Fluoroprobe in GMA. The results for this system are shown in *Table 3*. The differences between the poly-GMA material on the one hand and the core–shell material discussed above are clear from comparison of *Tables 2* and *3*. The fluorescence lifetimes in poly-GMA are significantly longer than in the latices. The lifetime distribution of Fluoroprobe fluorescence in the poly-GMA matrix, again, is much more narrow than in the core–shell latex material. In *Table 3* fluorescence lifetimes determined for Fluoroprobe in GMA at different emission wavelengths are given. Lifetime distributions are depicted in *Figure 4*. The trend, again, is a slight tendency to longer lifetimes with longer emission wavelengths. At the same time the contribution of the short lifetime (0.5–1.2 ns) component is diminishing. The short lifetime component may be due to the fluorescence from the Fluoroprobe

Table 1 Fluorescence lifetimes recovered for Fluoroprobe by NLLS analysis

Sample	λ_{em} (nm)	τ_1 (ns)	C_1 [a]	τ_2 (ns)	C_2	τ_3 (ns)	C_3	χ^2
Toluene	470 ± 10	8.2	1					3
Toluene/N_2	470 ± 10	12.3	1					7
Polystyrene	420 ± 10	1.5	0.31	7.1	0.69			36
		1.2	0.20	3.5	0.26	8.1	0.54	33
	> 450	3.4	0.26	12.2	0.74			125
		2.4	0.12	6.1	0.31	13.6	0.57	111
	470 ± 10	2.5	0.21	10.3	0.79			24
Polystyrene/GMA	> 370	1.4	0.27	7.1	0.73			56
		0.7	0.13	4.3	0.59	12	0.28	3
	420 ± 10	2.2	0.43	14.1	0.57			210
		1.2	0.22	7.2	0.63	179	0.15	2
	> 450	1.5	0.33	9.1	0.67			14
		0.9	0.20	5.2	0.53	15.9	0.27	1

[a] C_1 denotes fraction of the fluorescence intensity with fluorescence lifetime τ_1.

Table 2 Fluorescence lifetimes recovered for Fluoroprobe in latex particles by MEM

Sample	λ_{em} (nm)	τ_1 (ns)[a]	C_1[b]	τ_2 (ns)	C_2	τ_3 (ns)	C_3	τ_4 (ns)	C_4
Polystyrene	420 ± 10			1.1 [0.2]	0.14 [0.04]	4.5 [0.5]	0.48 [0.08]	10.9 [1.0]	0.38 [0.08]
	> 450			1.5 [0.1]	0.06 [0.01]	5.2 [0.7]	0.28 [0.09]	12.4 [1.2]	0.65 [0.10]
	470 ± 10			1.6 [0.7]	0.05 [0.04]			10.2 [0.9]	0.95 [0.04]
Polystyrene/GMA	370 ± 10	0.2 [0.2]	0.07 [0.03]	1.6 [0.4]	0.11 [0.04]	5.5 [0.4]	0.67 [0.07]	14 [2.4]	0.14 [0.06]
	420 ± 10	0.3 [0.2]	0.10 [0.02]			3.1 [0.4]	0.30 [0.06]	9.1 [1.4]	0.56 [0.07]
	> 450	0.4 [0.2]	0.13 [0.03]					8.2 [1.0]	0.85 [0.04]

[a] Number between brackets denotes the standard error
[b] C_1 denotes fraction of the fluorescence intensity with fluorescence lifetime τ_1

decomposition product at 370–400 nm, which is clearly observed and increases in intensity during the radical polymerization of GMA. Also in the core–shell latex a minor, short lifetime component is observed.

Time-resolved measurement of fluorescence anisotropy

As stated in the introductory section, fluorescence anisotropy measurements provide an insight into polymer dynamics, but the yardstick used in the measurement is the fluorescence process itself. For the present system this implies that the anisotropy decay can be measured over a limited period of time (about 10 ps to 50 ns), where the instrumental characteristics determine the low end and the decaying fluorescence intensity the high end of the range. In addition, the three fluorescence lifetimes are each expected to have their own fluorescence anisotropy decay,

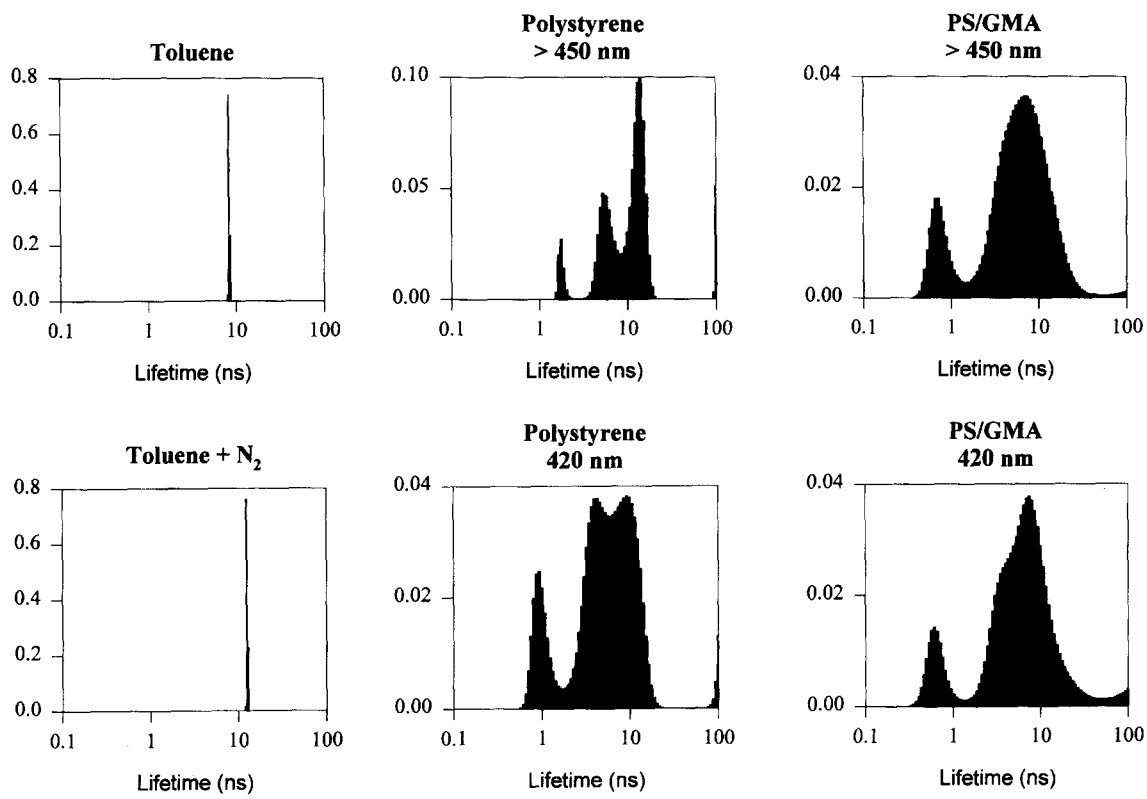

Figure 3 Fluorescence lifetime distributions obtained by evaluation of frequency-domain fluorescence measurements, analysed by the Maximum Entropy Method. Shown are data for Fluoroprobe in toluene (with and without deoxygenation) and for Fluoroprobe in polystyrene latex and in polystyrene/GMA core–shell latex

Table 3 Fluorescence lifetimes recovered for Fluoroprobe in polystyrene and polyglycidylmethacrylate by MEM

Sample	λ_{em} (nm)	τ_1 (ns)[a]	C_1[b]	τ_2 (ns)	C_2	τ_3 (ns)	C_3
Polystyrene	> 450	2.0 [0.4]	0.26 [0.09]	4.8 [0.8]	0.45 [0.09]	14.0 [1.9]	0.29 [0.12]
Poly-GMA	440 ± 10	1.2 [0.04]	0.44 [0.02]	4.6 [0.5]	0.28 [0.03]	15.8 [0.9]	0.28 [0.02]
	470 ± 10	1.0 [0.09]	0.29 [0.03]	4.9 [0.5]	0.46 [0.05]	16.1 [1.2]	0.25 [0.04]
	520 ± 10	0.59 [0.01]	0.19 [0.03]	5.1 [0.4]	0.59 [0.05]	16.0 [1.5]	0.22 [0.04]
	570 ± 10	0.59 [0.02]	0.16 [0.03]	5.2 [0.5]	0.64 [0.06]	16.6 [1.9]	0.20 [0.05]

[a] Number between brackets denotes the standard error
[b] C_1 denotes fraction of the fluorescence intensity with fluorescence lifetime τ_1

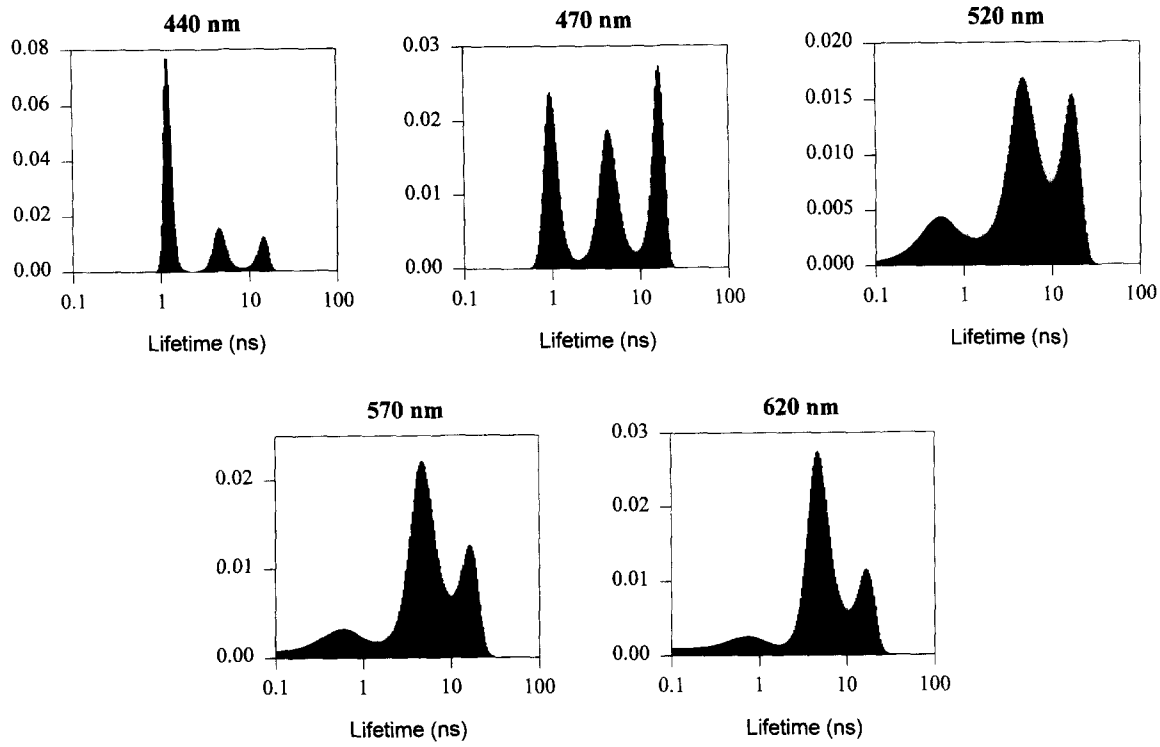

Figure 4 Fluorescence lifetime distributions obtained by evaluation of frequency-domain fluorescence measurements, analysed by MEM. Shown are data for Fluoroprobe in GMA, measured at a number of emission wavelengths

which makes analysis of the anisotropy data problematic. In fact, it appears that only anisotropy data obtained for Fluoroprobe in the polystyrene latex can be analysed with some confidence. The anisotropy decay in toluene solution is too fast for accurate measurement and the decays measured in the polystyrene film, in the core–shell latex or in the poly-GMA bulk polymer are either too complicated or too slow for straightforward analysis. The results obtained for Fluoroprobe in polystyrene latex are given in *Table 4*.

DISCUSSION

Steady state and time-resolved fluorescence

The fluorescence charge-transfer probes are complex compounds from the viewpoint of fluorescence lifetime methods: in high viscosity matrices they provide fluorescence lifetimes which are dependent on the emission wavelength (as is obvious when one considers the origin of the impressive solvent-induced shifts, see Introduction). In addition, the heterogeneity of the matrix adds to the complexity of the fluorescence lifetime data. The consequence of this complex picture is that one is forced to use the lifetime data in a qualitative way, treating the fluorescence lifetime distribution as a molecular fingerprint.

The analysis of complex, heterogeneous lifetime distributions is by no means an easy task. Efforts with NLLS render rather subjective results, which are not easily controlled. Introduction of lifetime distributions into the NLLS program led to unrealistic lifetimes. Better results were obtained with MEM. The very heterogeneous data fitted very well, and on the basis of the results of the fit procedure characteristics of the environment of the probe molecules can be made visible. The repeatability of the analysis is good, although in some cases the program appeared to have problems with apparently slightly separated lifetime peaks.

On the basis of the combined application of steady-state and time-resolved fluorescence data some conclusions can be drawn on the structure of the core–shell latex. A portion of the Fluoroprobe molecules is situated in the polystyrene core, which behaves similarly to the starting (bare) core material. The lifetime results (as well as the spectra, which are very similar for both types of matrices, cf. *Figure 1*), suggest that also in the core–shell latex, the Fluoroprobe is at least inside the polystyrene core. Another portion of the molecules occupies a place in a more immobile or apolar location in the latex, as is suggested by the blue shifted spectrum and the shorter lifetime of the Fluoroprobe[15]. On the other hand, the matrix is clearly not a poly-GMA one, since both

Table 4 Rotational correlation times recovered for Fluoroprobe in polystyrene latex particles by MEM

Sample	λ_{em} (nm)	τ_1 (ns)	C_1 [a]	r.c.$_1$ [b,c] (ns)	l.a.$_1$ [d]	τ_2 (ns)	C_2	r.c.$_2$ (ns)	l.a.$_2$
Polystyrene	420 ± 10	1.5	0.31	4.7 [0.5]	0.101 [0.001]	7.1	0.69	35 [3]	0.101 [0.001]
	> 450	3.4	0.26	5.5 [0.3]	0.094 [0.001]	12.2	0.74	47 [2]	0.094 [0.001]
	470 ± 10	2.5	0.21	7.1 [1.0]	0.093 [0.001]	10.4	0.79	48 [5]	0.093 [0.001]

[a] C_1 denotes fraction of the fluorescence intensity with fluorescence lifetime τ_1
[b] r.c., rotational correlation time
[c] Number between brackets denotes the standard error
[d] l.a., limiting anisotropy

fluorescence spectra and lifetime data are completely different for these two types of matrices. Alternative measurements (ζ-potential and field-flow fractionation measurements; J. W. Th. Lichtenbelt and A. van Asten, personal communication) indicate that the outer layer of the core–shell latex is homogeneous and contains a significant amount of water—the GMA is a very polar molecule. The fluorescence of Fluoroprobe is quenched in water[2], so that it can be assumed that any Fluoroprobe which is present in this phase is not observed in the emission signal. The significant difference in lifetime distributions between the Fluoroprobe in poly-GMA bulk probe on the one hand and the polystyrene–GMA latex on the other, leads to the conclusion that the Fluoroprobe in the shell does not experience a pure poly-GMA like environment. The shorter fluorescence lifetimes in the core–shell latex can be due to two phenomena: (1) interaction of Fluoroprobe with water, which may be in the shell, since GMA is rather polar, or close to the—very thin—shell; (2) the Fluoroprobe experiences a rather rigid environment which causes the spectrum to shift to the blue and the lifetimes to be shortened[15]. The second option seems to be the more probable one, also in view of the fluorescence spectrum of the core–shell latex which shows an additional band shifted to shorter wavelength. The presence of water would induce a red shift. The rigid matrix cannot be pure poly-GMA, in view of the differences between the data shown in *Table 3* for the fluorescence lifetime distributions and the fact that the fluorescence maximum of Fluoroprobe in poly-GMA is at about 536 nm (see *Figure 2*). The most likely second location of Fluoroprobe is in the interface between the polystyrene core and the glycidylmethacrylate shell, where a strongly interpenetrating macromolecular network is formed which may have a very strong rigidity and (in view of the presence of reacted polystyrene) a low polarity, in agreement with the observations.

Of course our interpretative possibilities are limited by the fact that we use an extrinsic probe: only parts of the latex which are accessible to the probe will be observed. At the same time the charge-transfer fluorescence probes cannot be used for the outer layers, since their fluorescence is quenched by protic solvents.

Time-resolved anisotropy

Time-resolved anisotropy is interesting for study of low molecular materials or, at least, low viscosity materials, unless long lifetime probes are applied. The fluoroprobe molecule has a lifetime of about 15 ns in medium polarity material. This figure indicates that relaxation phenomena occurring in the $> 1\ \mu$s timescale cannot be observed. In most materials studied in this paper anisotropy decays are too slow to be studied, or too quick (for the organic solvent system). Only in polystyrene in latex is some anisotropy decay observed. It is however, difficult to interpret the data. Apparently, the polystyrene latex is more flexible than the other materials. Swelling experiments have shown that solvents diffuse easily into the matrix (this is not the case in the polystyrene–GMA core–shell latex). Also the fluorescence lifetime results suggest a slightly higher mobility of Fluoroprobe in the polystyrene latex as compared to the polystyrene film.

The Fluoroprobe molecule shows a low limiting anisotropy; the most plausible explanation is that the transition dipoles of the S_0 and S_1 transitions are not parallel. An alternative explanation is that rapid energy redistribution takes place due to fluorophore–fluorophore interactions, which leads to a concomitant reduction of the limiting anisotropy.

In view of its short lifetime, its complex anisotropy decay and relatively low limiting anisotropy, the Fluoroprobe molecule does not appear to be a very suitable probe for dynamic fluorescence anisotropy measurements.

CONCLUSIONS

Fluorescent charge-transfer probes can be successfully used to obtain '*in-situ*' information on polymer structures and properties, and can be applied to following polymerization reactions. A disadvantage is the broad bands which are formed as a result of the combined action of fluorophore–solvent interactions and the heterogeneity of the polymeric matrix. This disadvantage is partially amended by the application of fluorescence lifetime analysis as an additional characterization parameter. The lifetime distributions are also heterogeneous, broad and wavelength dependent, which makes quantitative analysis difficult. The MEM appears to be well suited to unsupervised and objective analysis of these phenomenona. Via this approach, a qualitative 'fingerprint' picture is obtained, which allows for better founded interpretation of the spectral data. For analysis of rotational correlation times Fluoroprobe is less useful, because of its rather low limiting anisotropy and short lifetime, which limits the accessible range of rotational correlation times.

REFERENCES

1. Verhey, H. J., Gebben, B., Hofstraat, J. W. and Verhoeven, J. W., *J. Polym. Sci. Polym. Chem. Ed.*, 1995, **33,** 399.
2. Hermant, R. M., Bakker, N. A. C., Scherer, T., Krijnen, B. and Verhoeven, J. W., *J. Am. Chem. Soc.*, 1990, **112,** 1214.
3. Cundall, R. B. and Dale, R. E. *Time-resolved Fluorescence Spectroscopy in Biochemistry and Biology*. Plenum Press, New York, 1980.
4. Lakowicz, J. R., *Principles of Fluorescence Spectroscopy*. Plenum Press, New York, 1983.
5. Demas, J. N., *Excited State Lifetime Measurements*. Academic Press, New York, 1983.
6. McGown, L. B. and Bright, F. V., *Crit. Rev. Analyt. Chem.*, 1987, **18,** 245.
7. Brochon, J. C. and Livesey, A. K., *Chem. Phys. Letters*, 1990, **174,** 517.
8. Livesey, A. K. and Brochon, J. C., *Biophys. J.*, 1987, **52,** 693.
9. Skilling, J. and Bryan, R. K., *Mon. Not. R. Astr. Soc.*, 1984, **211,** 111.
10. Livesey, A. K. and Skilling, J., *Acta Cryst.*, 1985, **A41,** 113.
11. Gull, S. F. and Skilling, J., *IEEE Proc.*, 1984, **131F,** 646.
12. Siemiarczuk, A., Wagner, B. D. and Ware, W. R., *J. Phys. Chem.*, 1990, **94,** 1661.
13. Ugelstad, J., *Makromol. Chem.*, 1979, **180,** 734.
14. Gratton, E. and Limkeman, M., *Biophys. J.*, 1983, **44,** 315.
15. Hofstraat, J. W., Verhey, H. J., Verhoeven, J. W., Kumke, M. U. and McGown, L. B., in preparation.