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Excited-state probing of associative and covalent macromolecules

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Abstract.
The use of fluorescence spectroscopy in the study of associative phenomena is illustrated . The use of standard global of and global compartmental analysis in a number of complex systems is clearly shown. Association between detergent molecules and between detergents and polymers was analysed and the different parameters characterising such an interaction evaluated. Associative phenomena in covalent polymers such as dipole-dipole interactions in halato-telechelic polymers and in syndiotactic polymethylmethacrylate were investigated. The rate constants of inter and intramolecular interactions in halato-telechelic polymer were determined through a systematic analysis of the different interactions using global tricompartmental analysis of fluorescence decays combined with a scanning technique to estimate the rate constants. Fluorescence polarization and changes in fluorescence decay times in the temperature range of gelation reveal a stepwise mechanism in the gelation of s-PMMA.

INTRODUCTION

Traditionally luminescence spectroscopy has been used in the context of polymer chemistry to study intra and interchain complex formation between pendant aromatic groups intrinsic to the backbone of covalent macromolecules or to investigate excitation transfer in polymers in solution to gain information on chain dynamics. Luminescence spectroscopy has also been used to study free volume and free volume changes in polymer films.

In this contribution properties of singlet excited state of adequately chosen probes will be used to gain information on physical and chemical properties of macromolecules formed by associative or covalent bonding. Although it might at first sight look rather unorthodox to include micellar species in the realm of associative macromolecules it is clear , from the comparison with halato-telechic polymers (vide infra) , that the associative formation of micelles from detergent "monomers" can be compared to an associative polymerization. Fluorescence methods have been employed to describe static and dynamic properties of microstructures, such as surfactant systems forming micelles, surfactant-polymer systems where the surfactant and the polymer interact with each other, telechelic polymers and polymer systems forming polymeric aggregates and gels [1-3]. The methods take advantage of the fact that a probe molecule, which is excited by a light-pulse of suitable energy, can relax to its ground state by different radiative or non-radiative processes. According to which processes are at hand in a specific system, information about aggregate sizes, polydispersity, order parameters, equilibrium processes in the excited state, migration processes between and within the aggregates, and association processes can be extracted. In this contribution, we want to focus on some of the possibilities offered by the recent developments in the research group in the field of the dynamic fluorescence analysis to unravel detailed information on associative macromolecules and exemplify these with some recent experimental results.

Also associative phenomena in and covalent linking of polymers leading to either reversible or irreversible gelation can be probed and are in this contribution exemplified by dipole-dipole associations in halato-telechic polymers and by conformational change induced gelation in syndiotactic polymethylmethacrylate.

THE GLOBAL ANALYSIS AND GLOBAL COMPARTMENTAL APPROACH.

The most common way to compute estimates of model parameters is based on the non-linear weighted least-squares Marquardt [5] algorithm. The fits can be judged by a statistical residual analysis, e.g., the reduced χ_ν^2 and its normal deviate $Z(\chi_\nu^2)$ [4,6].
When analysing data in general, it has been shown that a global approach [7-9] provides better estimates of model parameters as compared to individual curve analysis. This is so not only for dynamic fluorescence data, but a general feature. Whenever it is possible to link at least one model parameter over different experiments one should simultaneously analyse the data. In the case of dynamic fluorescence measurements this is an obvious choice, as in most of the cases one or more of the parameters can be held common over the whole or a part of the experimental data surface. The recently developed compartmental analysis method of fluorescence decay surfaces [10] requires a global analysis approach. In a compartmental analysis, the system considered is treated as a system of different compartments for both the ground and the excited states (seeFig.1).

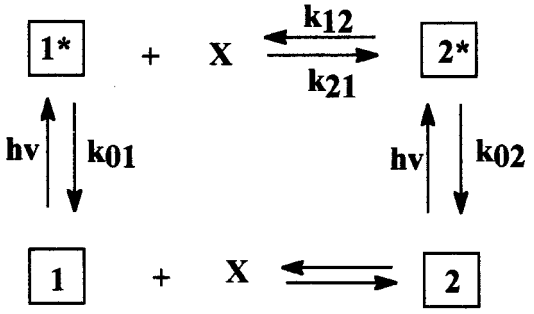

Fig.1 General Scheme for a two compartmental system

An aqueous micellar system, for example, can be considered as a compartmental system where the probe can be solubilized either in the micellar compartment (compartment **2**) or in the aqueous bulk compartment (compartment **1**). Compartmental analysis allows the determination of the rate constants for exchange between the different compartments, including the rate constants for fluorescence deactivation and quenching as well as spectral parameters.
Even if , based on the identifiability study of the system , not all parameters can be uniquely defined it is still possible to obtain upper and lower bounds for the system parameters [11].

ASSOCIATIVE POLYMERS: MICELLAR AGGREGATION AND POLYMER MICELLE INTERACTION

In a dynamic fluorescence quenching (DFQ) measurement a fluorescent probe molecule and a quencher molecule are added to the system of interest. The fluorescence decays of the probe in the presence of different concentrations of the quencher are recorded and analysed by appropriate kinetic models to recover the relevant parameters of the system. Two main situations can be distinguished, one with the probe molecule completely solubilized in the aggregate sub-phase and stationary in its host aggregate during the excited state lifetime of the probe, and in the other case one with the probe migrating between the aggregates during its excited state lifetime .
For micelles and similar aggregates, the basic relation is developed under the assumptions that the micellar aggregates are of equal size, that the fluorescence probe is stationary in its host micelle during the lifetime of the excited state, that the probe and the quencher molecules are Poissonian distributed among the micelles, and that the quencher molecules do not interact with each other [12].
In the case of an immobile quencher [12-13], i.e., the quenching rate is much faster than the quencher molecule exit rate, and the fluorescence decay rate is faster than the product of the quencher concentration in the bulk phase and the rate for quencher entry from the bulk into an aggregate, the fluorescence δ-response function takes a simple form,

$$F_t = A_1 \exp[-A_2 t - A_3\{1 - \exp(-A_4 t)\}] \qquad (1)$$

with the following expressions for A_1 - A_4:

$$A_1 = F_0 \quad (2a)$$
$$A_2 = k_0 \quad (2b)$$
$$A_3 = \langle n \rangle \quad (2c)$$
$$A_4 = k_q \quad (2d)$$

where F_0 is the intensity at time t=0, k_0 is the first-order rate constant for fluorescence deactivation in the absence of a quencher (k_0 is often given as the decay time τ_0; $\tau_0 = 1/k_0$), $\langle n \rangle$ is the average occupation number of quenchers in a micelle; $\langle n \rangle = Q_m / M$, with the subscript m denoting the micellar phase and M the micelle concentration, and k_q is the first-order rate constant for quenching in a micelle containing one quencher.

The immobility of the probe is one of the conditions in the original model. In many cases this is true, e.g., when a highly hydrophobic probe is introduced to an aqueous micellar solution of moderate micelle concentration. In other cases, however, this condition may not hold, as in the case of highly concentrated micellar solutions, reversed micellar solutions or when the probe has an amphiphilic character [14-17].

For the case of migrating probes and/or quenchers, generalised versions of the model have been developed [18,19]. In a first approach [18] it was shown that the fluorescence δ-response function will still be described by an equation similar to Eq. 1, but with a generalised interpretation of the A_i parameters. According to this approximate solution, the different A_i can be expressed as Eqs. 3 (A_1 is the same in all models):

$$A_2 = k_0 + \langle x \rangle_s k_q \quad (3b)$$
$$A_3 = \langle n \rangle (1 - \langle x \rangle_s / \langle n \rangle)^2 \quad (3c)$$
$$A_4 = k_q / (1 - \langle x \rangle_s / \langle n \rangle) \quad (3d)$$

where $\langle x \rangle_s$ is the long time value of $\langle x \rangle$, the average number of quenchers in a micelle with an excited probe, and all other parameters have the same meaning as above. It follows that the observed fluorescence decay becomes mono-exponential if, and only if, $\langle x \rangle$ reaches a constant value $\langle x \rangle_s$ within the time-window studied.

It has been shown that this approximation holds only at low average numbers of quencher per micelle, $\langle n \rangle$ [20]. Alternatively, on the basis of a stochastic approach, an exact solution for this problem was presented [19]. It could be shown that in this approach $\langle x \rangle_s$ can be evaluated by the following expression [19]

$$\langle x \rangle_s = (k_p M / k_q)\{1 - \exp[-A_3 A_4 / (A_4 + k_p M)]\} + \langle n \rangle k_{exq} / A_4 \quad (4)$$

where k_p is the second-order rate constant for the probe migration process and k_{exq} is the generalised quencher exchange rate constant. The product $k_p M$ can be regarded as the reciprocal of the first passage time between two micelles when migration through the bulk phase is considered [19].

From Eq. 4, Eqs. 3 can be rewritten [19,21,22] to give

$$A_2 = k_0 + k_p M\{1 - \exp[-\langle n \rangle k_q / (k_q + k_p M)]\} \quad (5b)$$
$$A_3 = \langle n \rangle \{1 - k_p M(1 - \exp[-\langle n \rangle k_q / (k_q + k_p M)]) / k_q \langle n \rangle\}^2 \quad (5c)$$
$$A_4 = k_q \{1 - k_p M(1 - \exp[-\langle n \rangle k_q / (k_q + k_p M)]) / k_q \langle n \rangle\}^{-1} \quad (5d)$$

or, when the probe is considered to be immobile;

$$A_2 = k_0 + \langle n \rangle k_{exq} / A_4 \quad (6b)$$
$$A_3 = \langle n \rangle k_q^2 / A_4^2 \quad (6c)$$
$$A_4 = k_q + k_{exq} \quad (6d)$$

which will reduce to Eqs. 3 at low $\langle n \rangle$-values.

The use of Eqs. 5 or 6 in the analysis of the fluorescence decay permits the determination of the different model parameters under the condition that both probe and quencher migrations are allowed.
The compartmental analysis method [10] was used to determine the distribution of naphthalene between cetyltrimethylammonium chloride (CTAC) micelles and the aqueous bulk phase [21].
The interactions between surfactants and polymers have been intensively studied by classical methods [22]. The use of dynamic fluorescence quenching, however, has appeared quite recently as method for the study of these kind of systems [21,23,24], and has become an important tool in this field. The interactions between polyelectrolytes and surfactants are in principle determined by the balance between electrostatic and hydrophobic forces, i.e., the interaction between the polyelectrolyte and the surfactant starts as a counterion binding of the surfactant to the polyelectrolyte, while the driving force for aggregate formation is hydrophobic. For systems containing a nonionic polymer, the hydrophobic force is the most important. It is well-known that the interactions between anionic surfactants and nonionic polymers are much stronger as compared to systems with a cationic or nonionic surfactant [22].
It was, however, shown in a DFQ study of the cationic surfactant CTAC and the nonionic poly(vinyl alcohol)-poly(vinyl acetate), PVA-Ac, that also cationic surfactants interact with nonionic polymers [21]. The interaction caused the formed aggregates to have a smaller aggregation number as compared to ordinary CTAC micelles. It was also concluded that the polymer strand coils around the aggregates in very much the same way as has been suggested for anionic surfactants interacting with nonionic polymers [24b]. The association of ionic surfactants to the polymer causes the polymer-surfactant complex to behave more and more as a polyelectrolyte, as confirmed by viscosity measurements.
With the use of compartmental analysis [10], it was possible to evaluate the solubility capacity of the surfactant aggregates. Introducing PVA-Ac to an aqueous CTAC solution increases the solubility capacity of small, hydrophobic, molecules. The equilibrium constant for partitioning between aggregates and the aqueous bulk increased by approximately 50% when the polymer was introduced. This was rationalised by the reduced polarity of the aggregate Stern layer, a reduction also monitored by the ratio of the first and third vibronic peaks in the pyrene steady-state emission spectrum.

Table 1 Migration of Pyrenesulphonic acid in CTAC in absence and presence of PVOH

	CTAC 0.007 M			CTAC 0.01 M		CTAC 0.02 M	
PVOH		+0.5 gr/dl	+1 gr/dl		+1 gr/dl		+1 gr/dl
k_0	7900	7900	7900	8000	7900	7900	7900
k	*	**8**	**3.8**	**0.5**	**6**	**1.54**	**3.8#**

rate constants in 10 E6 sec,* no exchange, # not all detergents associated to a polymer chain

The presence of a polymer in a micellar solution may also alter the migration rates.(table 1). It was found that, based on global analysis of the fluorescence decay of PSA in the presence of the quencher TPyrC, the probe migration increased by the presence of the polymer compared with a polymer-free solution at the same surfactant concentration. Furthermore, increasing the polymer concentration, at a fixed surfactant concentration, leads to a decrease in migration rate and, equivalently, increasing the surfactant concentration at constant polymer concentration increases the migration rate. This was accounted for by the higher local concentration of micelles interacting with a polymer chain.

COVALENT POLYMERS: ASSOCIATION IN HALATO-TELECHELIC POLYMERS AND IN S-POLYMETHAMERTHYLACRYLATE

Assocatiation in halato-telechic polymers can be probed by stationary fluorescence spectroscopy if the end groups of the polymers are capped with adequate probe groups [25]. We have shown some time ago that pyrene endcapped halato-telechelic polymers reveal through the ratio of excimer to monomer emission (Fig.2) competion between intra and intermolecular associations . This was shown by thi ratio as a function of the polymer concentration of an added polymer of identical molecular weight but containing no photophysical. probe (Fig. 3)

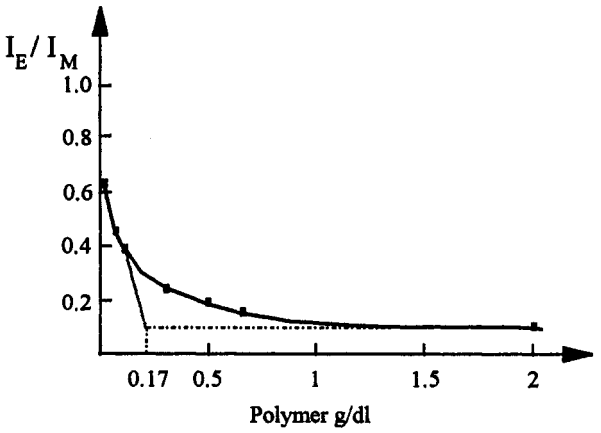

Fig.2 Excimer to locally excited state emission in pyrene endcapped halato-telechelic polymer (ref.25)

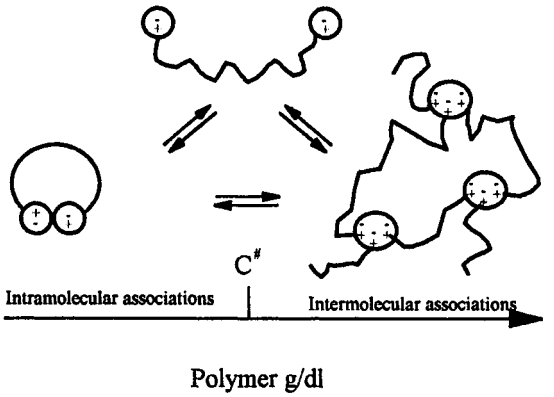

Fig. 3 Competition between inter and intramolecular association in halato- telechelic polymers

This observatiuon based on stationary fluorescence could be qualitatively interpreted by a scheme represented in Fig. 3.

Global compartmental analysis combined with a scanning technique [11] has now allowed us to estimate the different rate constants involved in this rather complicated system. The following strategy was used to unravel the system. First the photophysiscs were investigated of N,N,N-trimethyl-3-(1-pyrenyl)-1-propanaminium perchlorate (PROBE) in THF at room temperature. This represents the end cap of the polymer chain . Excimer formation had to be considered according to the routes depicted in Fig.4:

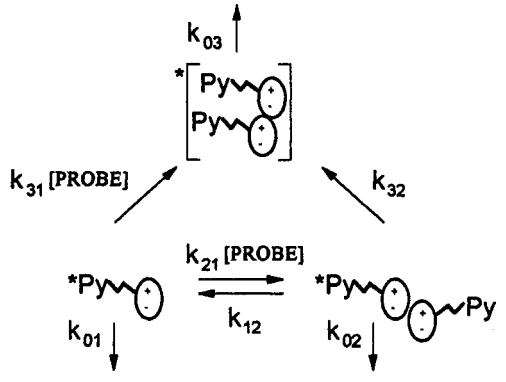

Fig. 4 Intermolecular association of PROBE in THF at room temperature

At very low concentrations PROBE decays monoexponentially with a lifetime τ of 236 ± 1 ns, from which $k_{01} = 1/\tau = 4.2 \times 10^6\ s^{-1}$ is obtained. Upon addition of the quaternary ammonium salt N,N,N-trimethyl-1-dodecanaminium perchlorate a biexponential decay function is needed to describe the decay traces. The second excited state is the aggregated PROBE. This aggregation is due to dipole-dipole or ion-dipole interactions. The rate constant values of the kinetic scheme (Fig. 4) are obtained by global bicompartmental analysis: $k_{01} = k_{02}$, $k_{21} = (42 \pm 7) \times 10^9\ M^{-1}s^{-1}$, $k_{12} = (5.7 \pm 0.1) \times 10^7\ s^{-1}$. When the concentration of PROBE itself is varied, now a triple exponential decay function adequately describes the decay surface. The third excited-state species is a PROBE excimer, which can be formed through two different pathways: either intermolecularly when a locally excited PROBE molecule encounters a ground-state PROBE molecule or intramolecularly when an aggregate of two PROBE molecules rearranges. To resolve the kinetics of this system global tricompartmental analysis is developed. Even after including the information available from experiments where N,N,N-trimethyl-1-dodecanaminium perchlorate is added ($k_{01} = k_{02}$), and the information available from the global triple exponential analysis ($k_{13} = 0$ and $k_{23} = 0$) the experimental time-resolved data do not allow one to obtain a unique solution for the rate constant

values. By applying the scanning technique [11], upper and lower bounds can be specified for the constants: $53 \times 10^9 < k_{21} < 60 \times 10^9$ $M^{-1}s^{-1}$, $k_{31} < 7 \times 10^9$ $M^{-1}s^{-1}$, $1.5 \times 10^8 < k_{12} < 1.7 \times 10^8$ s^{-1}, and $k_{32} < 2 \times 10^7$ s^{-1}. Unique values are obtained for k_{01}, k_{02}, and k_{03}: $k_{01} = k_{02} = (4.25 \pm 0.01) \times 10^7$ s^{-1}, $k_{03} = (1.92 \pm 0.03) \times 10^7$ s^{-1}.

In a second step the photophysics of POLYPROBE , a polymer chain of well defined length ending with a group identical with PROBE was studied in THF at room temperature. The following processes had to be considered analogous to those observed for PROBE:

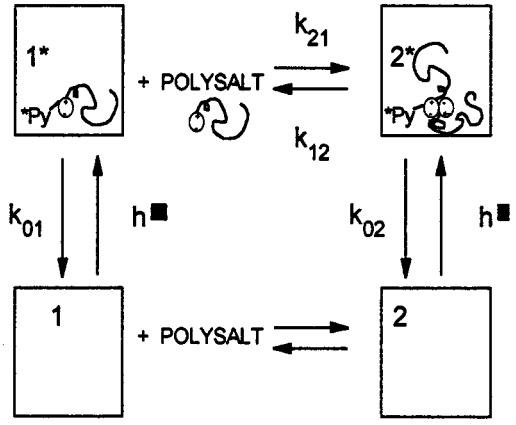

Fig.5 Interaction between POLYPROBE and Polysalt in THF at room temperature

Analysis of the fluorescence decays allowed us to determine the following rate parameters (Table 2):

Table 2 Rate parameters for the interaction between POLYPROBE and Polysalt in THF at room temperature.

$k_{01} = (4.38 \pm 0.05) \times 10^6$ s^{-1}	$k_{02} = (6.66 \pm 0.07) \times 10^6$ s^{-1}
$k_{21} = (13 \pm 2) \times 10^9$ M^{-1} s^{-1}	$k_{12} = (12 \pm 1) \times 10^7$ s^{-1}

Table 3 Rate Parameters in THF at room temperature

	PROBE	PROBE/ DOTAP	POLY-PROBE	POLYPROBE/ POLYSALT
$k_{21} / 10^9$ M^{-1} s^{-1}	53 - 60	42	17.2 - 20	13
$k_{31} / 10^9$ M^{-1} s^{-1}	< 7		< 2.7	
$k_{12} / 10^9$ s^{-1}	0.15 - 0.17	0.057	0.13 - 0.145	0.12
$k_{32} / 10^9$ s^{-1}	< 0.02		< 0.02	
K^* / M^{-1}	312 - 400	700 ± 130	120 - 158	108 ± 25

* dimensions of k_{21} and k_{31} in s^{-1}.

In a following step the POLYPROBE-POLYPROBE interactions were investigated according to the scheme reported in Fig. 6

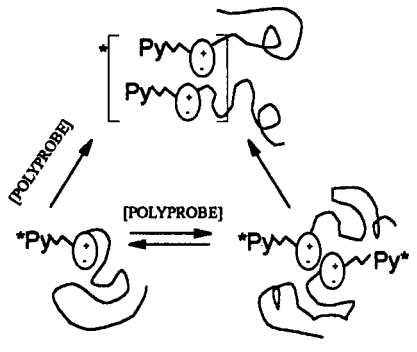

Fig.6 POLYPROBE-POLYPROBE interactions in THF at room temperature

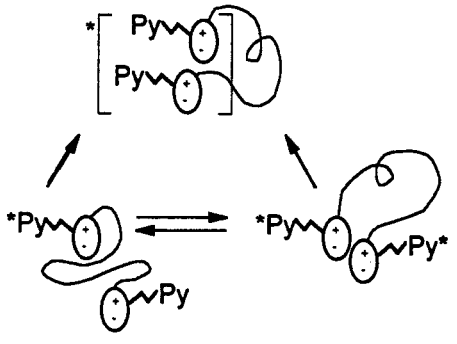

Fig. 7 Intramolecular interactions in POLYDIPROBE in THF at room temperature

The analysis of the fluorescence decays using global tricompartmantal analysis allowed us to estimate the rate parameters reported in Table 3. In a final step the intramolecular dipole-diolpe association in competion with intramolecualr excimer formation in POLYDIPROBE, a polymer with identical cahin length as POLYPROBE but capped at both end with a probe group was investigated and analysed according to the scheme represented in Fig. 7.

Fluorescence spectroscopy offers the possibility to contribute to the elucidation of association phenomena in covalent polymers such as s-polymethylmetacrylatye. This technique was already used previously to study polymer conformation change and thermoreversible gelation [26,27]. Because sPMMA has no intrinsic chromophores, the polymer was labelled in these experiments with a fluorescent molecule. sPMMA, containing 1% 1-pyrenemethylmethacrylate as a comonomer, has been synthesized. An average of four labelled monomer units per polymer chain has been calculated from absorption measurements. This copolymerization does not influence the tacticity and the molecular weight of the polymer. A low molecular weight material, 1-pyrenylpivalate, was used as a model compound in the analysis.

The fluorescence spectra of the model compound is influenced by the concentration. At high concentrations a broad structureless band appears with a maximum at 485 nm which is ascribed to excimer formation. This band is absent at low concentration. This concentration dependence of the intensity of the excimer formation is not observed with the labelled polymer. In this case the local concentration of chromophores must always be relatively high and excimer formation is observed at any polymer concentration. The fluorescence decay of a solution of sPMMA*-syndiotactic PMMA containing a labael pyrene- in toluene at 377 nm can be analysed a sum of two exponential functions. The longer lived component of the emission can be attributed to chromophores that cannot form an excimer during the excited state lifetime. The shorter lived emission originates from chromophores at excimer-forming sites. This attribution is supported by the analysis of the fluorescence decay at 500 nm. This kinetic scheme has been further confirmed by using a polymer with a lower concentration of built-in chromophores (0,1%). No excimer emission is observed in the fluorescence spectra of a solution of this polymer and a monoexponential fluorescence decay with a decay time of 212 ns is observed for a gel with w_2=0.05. This proves that the biexponential fluorescence decay is a consequence of the `intrachain` excimer formation.

The contribution of the excimer emission to the biexponential fluorescence decay is limited because of the low concentration of these chromophores along the polymer chain. Consequently the longest component in the decay has been used in this study of the gelation. This has been justified by a limited number of experiments using a sPMMA with 0.1% pyrene labels that did not show eximer formation and for which the decay corresponds to this longest decay component.

The change in chain conformation is studied by measuring the fluorescence decay of the probe at different temperatures. In a first series of experiments a dilute solution with a polymer concentration w_2=0.005 has been used. Under these conditions no macroscopic gel is formed. When the solution is cooled, a sharp increase in the decay time, extending over only a few degrees, sets in around 50°C. This change is situated in the temperature range where the first traces of conformational change are observed by IR. The recorded values represent equilibrium data at each temperature. The recorded value is obtained as soon as the sample reaches the desired temperature and no further change with time was observed. An increase in the solute concentration (w_2=0.05) only displaces the onset of this transition to higher temperature without influencing its position as a whole on the temperature scale. This sudden change in decay time is not observed for a solution in toluene of the atactic, labelled PMMA. A linear decrease is observed over the whole temperature range studied.

The change in fluorescence characteristics can be related to the change in chain conformation. In a random coil, the chromophores are predominantly surrounded by polar ester groups. This results in a lower fluorescence decay time. The transition form this random coil to a helix conformation of some of the molecular segments will expand the chain molecule. The less polar solvent, toluene, will replace the more polar ester groups and the polarity in the vicinity of the chromophore will decrease. This leads to an increase in the fluorescence decay time.

The transition of a few segments of the chain from the coil to the helix conformation seems to be sufficient to realize this change in solvatation of the pyrene groups. A study of the helix-coil transition of the 1-pyreneacrylic acid-methacrylic acid copolymer as a function of the pH has led to similar conclusions [28].

A study of stationary fluorescence anisotropy of the pyrene label provides additional information on the thermal transitions that take place on gelation and gel melting. A solution of sPMMA* in toluene with a w_2=0.1 shows no fluorescence anisotropy at temperatures above the gel temperature. However, upon cooling the solution, fluorescence anisotropy is observed at temperatures below the gelation temperature. At low temperature the solution is rigid and the fluorescence of the probe shows anisotropy. The gel melts when reheating and this results in the disappearance of the physical network and an important decrease in the fluorescence anisotropy in the temperature region in which a transition is observed by IR, DSC en ^{1}H NMR.

A dilute solution of sPMMA* in toluene with a concentration of w2=0.001 shows no fluorescence anisotropy, neither do solutions of labelled aPMMA at any concentration in the same temperature region, clearly indicating that network formation and not conformational change leads to this fluorescence anisotropy.
A very large hysteresis , which is a function of the cooling rate , is observed. This is due to the fact that the values for the fluorescence anisotropy in this experiment are no equilibrium values. The changes in fluorescence anisotropy of a w2=0.1 solution in toluene of sPMMA* at 35°C as a function of time clearly shows that 5 to 6 hours are needed before equilibrium is reached at that temperature.
From this evidence a stepwise gelation of s-polymethylmethacrylete can be postulated.

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