RECENT APPLICATIONS OF NONRADIATIVE ENERGY TRANSFER TO POLYMER STUDIES

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Dedicated to Professor Otto Wichterle on the occasion of his 80th birthday.

Recent studies of polymers in solution and in bulk by energy transfer between two fluorescent labels are reviewed. Such studies are concerned with the equilibrium and dynamics of polymer chain expansion, molecular cluster formation in solution, the miscibility of polymers in bulk, and the interdiffusion of polymer latex particles.

In studies of biological macromolecules, fluorescence has long been recognized as an important experimental tool[1]. Of various phenomena involving fluorescence, one of the most valuable is nonradiative energy transfer (NET) which is observed in systems containing two fluorophores such that the emission spectrum of one (the donor) overlaps the absorption spectrum of the other (the acceptor). According to the theory proposed by Förster[2,3], the efficiency of the transfer of the excitation energy from the donor to the acceptor, E, is given by

$$E = R_0^6/(R_0^6 + r^6) \tag{1}$$

$$R_0^6 = (8.8 \cdot 10^{-25}) J \, n^{-4} \, \kappa^2 \, \varphi_D^0 , \tag{2}$$

where r is the distance between donor and acceptor, R_0 is a characteristic distance at which half of the excitation energy is transferred, J is the overlap integral between the emission spectrum of the donor and the absorption spectrum of the acceptor, n is the refractive index, κ^2 is a function of the mutual orientation of the donor and acceptor transition moments, and φ_D^0 is the fluorescence quantum yield of the donor in the absence of acceptor. Thus, NET may be employed to estimate the distance between two labels and it has, therefore, been referred to as a "spectroscopic ruler"[4]. The major difficulty in its use is due to the uncertainty of κ^2, which may assume values from 0 to 4 when the donor and acceptor are both attached to a relatively rigid structure such as

a globular protein. This problem may be overcome by combining NET data with data on the depolarization of fluorescence[5]. The early use of NET for the study of biological macromolecules has been reviewed by Steinberg[3]; recently, this phenomenon has been exploited in studies of the mechanism by which proteins fold, after biosynthesis, to their native globular structures[6,7].

In synthetic polymer studies, NET has first been used for the characterization of the miscibility of blends of donor and acceptor-labeled polymers[8 – 10]. In these and later studies, the systems contained small concentrations of the fluorophores whose mutual orientation could be considered random. This introduces a great simplification into the interpretation of data, since κ^2 assumes then the value of 2/3 in fluid media[2,3] and a value of 0.475 in rigid systems in which the rotational diffusion constant of the labels is small compared to the rate constant for energy transfer[11]. A review is available summarizing polymer studies up to 1988 employing NET (ref.[12]); here I wish to discuss recent developments in this field.

Extension of Polymer Chains

Liu et al. have characterized by NET the end-to-end displacement, r, of polymer chains with a donor and acceptor chromophore attached to the terminal monomer residues[13 – 15]. This approach is, of course, only feasible when r is not much larger than R_0 of the fluorescent labels used (R_0 is usually less than 3 nm). Thus, the technique is limited to vinyl polymers with degree of polymerization DP $\approx$ 100. In the study involving poly(methyl methacrylate)[13], NET data on the end-labeled polymer incorporated in an unlabeled matrix yielded chain extensions in good agreement with values deduced by other techniques. However, when PMMA (ref.[13]) or polyelectrolytes[14,15] were studied in solution, the chain dimensions estimated from NET were too low. This discrepancy is due to the neglect of chain dynamics. Since the energy transfer efficiency increases rapidly with a decreasing separation of donor and acceptor, this efficiency will increase if the chain ends can diffuse towards each other during the excited state lifetime of the donor. This effect results in a decreasing apparent distribution function of chain end separations as the fluidity of the system is increased[16].

When polymers are doubly labeled with donor and acceptor fluorophores distributed at random along the molecular backbone, the chain extension may be followed by NET in chains of any length, although the interpretation of the data is more uncertain than with end-labeled polymers. Randomly doubly labeled poly(N-isopropylacrylamide), which is known to precipitate from aqueous solution when heated above 31 °C, exhibited a smooth increase in energy transfer below the temperature of phase separation, but a less rapid increase, reflecting a continuing chain contraction, was observed even above that temperature[17]. Chain dynamics can also be studied using polymers carrying two fluorophores. Here randomly doubly labeled poly(methacrylic acid) was mixed in a stopped-flow apparatus with buffers of a pH at which the PMA is ionized to varying

degrees and the chain expansion was monitored by the decreasing acceptor fluorescence on irradiation in the donor absorption band[18]. Although the initial phase of the expansion was too fast for this technique, about 60% of the process could be followed. The final approach to conformational equilibrium was surprisingly slow, a phenomenon which is not understood.

Molecular Association in Polymer Solutions

As a polymer solution approaches the temperature of phase separation, the concentration of the chain molecules, referred to in the last section, is accompanied by an association between the macromolecular chains. This process was studied by NET on solutions of poly(N-isopropylacrylamide) where half of the polymer was labeled with a donor and half with an acceptor fluorophore[19]. Such clustering of polymer chains is also characteristic of aqueous solutions of polymers carrying hydrophobic substituents, e.g., hydroxypropyl-cellulose, and it can again be studied by this method[20]. A special case of polymer aggregation is observed in solutions of diblock copolymers in media in which only one block is solvated. In that case, micelles are formed with the dense core containing the precipitated blocks surrounded by a halo of the solvated blocks. Two laboratories reported NET studies of systems of this kind. In the first one, styrene–isoprene diblock copolymers with either block terminally labeled with donor or acceptor were employed[21]. When the composition of the solution containing a mixture of block copolymers labeled in the same block with either the donor or the acceptor was adjusted so as to desolvate the labeled blocks, a sharp increase in NET was observed, reflecting the polymer micelle formation. Attempts to demonstrate a critical micelle concentration were inconclusive, although micelle formation was clearly favored by an increasing chain length. The NET data indicated also micelle formation when the block copolymer was blended with polyisoprene, but there was again no evidence of a critical micelle concentration. The second study was of particular interest in that it included data on the kinetics of polymer interchange between donor and acceptor labeled micelles[22]. This process is extremely slow, requiring typically an hour for approach to equilibrium. Its rate decreases sharply as the medium becomes a stronger precipitant for the blocks forming the core of the micelles.

A special case of polymer association is the stereocomplex formation from isotactic and syndiotactic poly(methyl methacrylate)[23] and recently NET has also been used for the study of this process[24]. It confirmed the dependence of the association on the solvent and the stoichiometry of the stereocomplex deduced from results obtained by other experimental methods.

Polymer Miscibility

When Amrani et al.[8] first introduced energy transfer in mixtures of donor and acceptor labeled fluorophores as a technique to characterize polymer miscibility, they made it clear that a quantitative interpretation of the data is ambiguous, since the efficiency of NET should be sensitive not only to the extent of phase separation, but also to the dimensions of the phase domains. In more recent work it has been pointed out that special care has to be taken to estimate the extent of energy transfer corresponding to complete phase separation[25]. Moreover, it was also shown[26] that when the donor and acceptor labeled polymers are diluted by unlabeled polymers (as is customarily done so as to arrive at blends with the desired label concentration), NET for any overall label concentration will depend on the fluorophore content of the labeled chain molecules.

When such complications are taken into account, NET was demonstrated to be valuable as a tool for the study of polymer miscibility. In mixtures of poly(vinyl chloride) with poly(methyl methacrylate) of varying tacticity, NET indicated no phase separation but a sharp increase of donor fluorescence when comparing blends containing 10% of PMMA with energy transfer of donor and acceptor labeled PVC (ref.[25]). This was interpreted as indicating that PVC acts as a quencher and that contact with PMMA protects the donor from quenching. On this basis the experimental data suggested that the effectiveness of such protection increases with the isotacticity of the PMMA, where polymer heterocontacts are most favored.

Another application concerns the study of polymer modification to improve miscibility. Polystyrene is incompatible with polymethacrylates, but NET data on donor and acceptor labeled polymer blends[27] demonstrated that the incorporation of as little as 2 mole % of p-vinylphenol or p-(hexafluoro-2-hydroxylisopropyl)styrene (HHIS) residues into the polystyrene chain was sufficient to effect miscibility as a result of hydrogen bonding with the polymethacrylate ester groups. More recently it was shown[28] that energy transfer continues to increase with the HHIS content of the polystyrene beyond the point where transmission electron microscopy indicates that the blend with poly(methyl acrylate) is homogeneous, suggesting an increasingly intimate contact between the components of the blend.

Latex Particle Studies

In time resolved fluorescence studies of rigid systems with randomly distributed donor and acceptor fluorophores, the observed decay of the donor emission intensity reflects the distribution of donor–acceptor distances. If one of the dimensions of the phase is comparable to the characteristic energy transfer distance (R_0) in Eq. (2), the kinetics in this "two-dimensional space" acquire a characteristic form[29]. Such a situation was encountered in the study of latex particles obtained by polymerizing vinyl acetate in isooctane solution containing poly(2-ethylhexyl methacrylate) (PEHMA)[30]. The PVAc

particles were found to be penetrated by a PEHMA network and when this network was doubly labeled with donor and acceptor fluorophores, the donor fluorescence decayed, after flash irradiation, as expected in a two-dimensional space. Using the same technique with a film cast from this material, the kinetic results corresponded to an average dimensionality of 1.4, suggesting that the PEHMA was present in the film in the form of interconnected thin cylinders[31].

A process of great technological importance for the paint industry is the coalescence of latex particles to form a continuous polymer film[32]. This has been studied most efficiently by following the decay of donor fluorescence after a flash excitation during the interdiffusion of donor and acceptor labeled poly(butyl methacrylate) (PBMA) latex[33]. The temperature dependence of the apparent diffusion coefficient was in good agreement with that derived from dynamic mechanical measurements of PBMA.

Concluding Remarks

This review demonstrates that a variety of problems in the physical chemistry of polymers can be elucidated by NET. It should be noted that very small concentrations of fluorophores are sufficient, so that the presence of the labels will generally not lead to a significant alternation of the properties of the system. An exception may be encountered with a hydrophilic polymer in aqueous solution, where even small numbers of hydrophobic labels may lead to their intramolecular and intermolecular aggregation.

Although steady-state fluorescence measurements are generally adequate for yielding the desired information, time-resolved studies of donor emission have the important advantage of being relatively insensitive to radiative energy transfer which does not depend on the donor–acceptor separation as given by Eq. (2) (ref.[34]).

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