

# Intermolecular Foerster's Energy Transfer in Labeled Poly[*N*-(2-Hydroxypropyl)methacrylamide]

Drahomír Vyprachtický,<sup>1</sup> Veronika Pokorná,<sup>1</sup> and František Mikeš<sup>1</sup>

Received October 18, 1993

By copolymerization of monomers containing donor (carbazole) and acceptor (dansyl) fluorophores with *N*-(2-hydroxypropyl)methacrylamide (HPMA), statistical copolymers with "low" and "high" contents of the fluorophores were prepared. The increase in nonradiative energy transfer between copolymers with a low content of fluorophores was probably due to intermolecular penetration of the polymer coils in concentrated solutions.

**KEY WORDS:** Poly[*N*-(2-hydroxypropyl)methacrylamide]; Foerster's energy transfer.

## INTRODUCTION

Electronic excitation energy may be transferred from a donor to an acceptor fluorophore through an induced resonance interaction if the emission spectrum of the donor overlaps the absorption spectrum of the acceptor. A theoretical basis for resonance nonradiative energy transfer (NET) was derived by Foerster [1,2] by means of dipole-dipole approximation of Coulombic interactions. The rate of the transfer was predicted to be inversely proportional to the sixth power of the distance between the interacting fluorophores and thus very sensitive to this distance. The NET has been used extensively to characterize distances in biological macromolecules [3,4] and synthetic polymers [5,6], and the phenomenon was also used in the investigation of polymer blend compatibility [7,8].

## EXPERIMENTAL

### Materials

9-(3-Methacryloylaminopropyl)carbazole (MAPC) was prepared by the reaction of 9-(3-aminopro-

pyl)carbazole [9] with methacryloyl chloride in the presence of triethylamine. *Anal. for* C<sub>19</sub>H<sub>20</sub>N<sub>2</sub>O (292.38). Calc: 78.05% C, 6.89% H, 9.58% N. Found: 78.07% C, 6.89% H, 9.33% N. The structure of MAPC was confirmed by IR and NMR spectra.

5-Dimethylamino-1-(2-methacryloylaminoethyl)naphthalenesulfonamide (Dns-MA) was prepared by the reaction of *N*-(2-aminoethyl)-5-dimethylamino-1-naphthalenesulfonamide with methacryloyl chloride [10]. *N*-(2-Hydroxypropyl)methacrylamide (HPMA) was prepared by a known procedure [11].

The copolymers of monomers MAPC and Dns-MA with HPMA (**I** and **II**, respectively) with different contents of the fluorophores (a, b) were prepared by radical precipitation polymerization in acetone. In all cases the total monomer concentration was 15 wt%, the temperature 60°C, and the reaction time 2.5 h. Copolymers **Ia**, **Ib**, **IIa**, and **IIb** are characterized in Table I.

### Fluorescence Measurements

Efficiency of NET ( $Q_{ET}$ ) from carbazole to dansyl fluorophore was studied by means of reflectance fluorescence spectroscopy (30°/60°). The carbazole fluorophore was excited at 294 nm and its emission was recorded

<sup>1</sup> Institute of Macromolecular Chemistry, Academy of Sciences of the Czech Republic, Heyrovský Sq. 2, 162 06 Prague 6, Czech Republic.

**Table I.** Characterization of Copolymers I and II with a High (a) and a Low (b) Content of Fluorophores

Copolymer	Monomer label	AIBN (wt%) <sup>a</sup>	Total convers. (wt%)	[ $\eta$ ] (dl g <sup>-1</sup> ) <sup>b</sup>	Label content (mol%)	
					Feed	Copolymer
Ia	MAPC	0.20	10	0.56	5	2.3
Ib	MAPC	0.12	12	1.28	2	0.9
IIa	Dns-MA	0.20	11	0.32	5	3.0
IIb	Dns-MA	0.12	14	0.41	2	1.2

<sup>a</sup>Per mixture of monomers.<sup>b</sup>In water at 25°C.

at 365 nm. The efficiency of NET was calculated from the ratio of the donor emission intensity in the presence  $I_D(\lambda)$  and absence  $I_D^0(\lambda)$  of acceptor, after correcting this ratio for the screening effect of the acceptor, as

$$\left[ \frac{I_D(365)}{I_D^0(365)} \right]_{\text{corr.}} = \frac{I_D(365)}{I_D^0(365)} \frac{\epsilon_{294}^D \cdot c^D + \epsilon_{294}^A \cdot c_A + \epsilon_{365}^A \cdot c_A / \sqrt{3}}{\epsilon_{294}^D \cdot c_D}$$

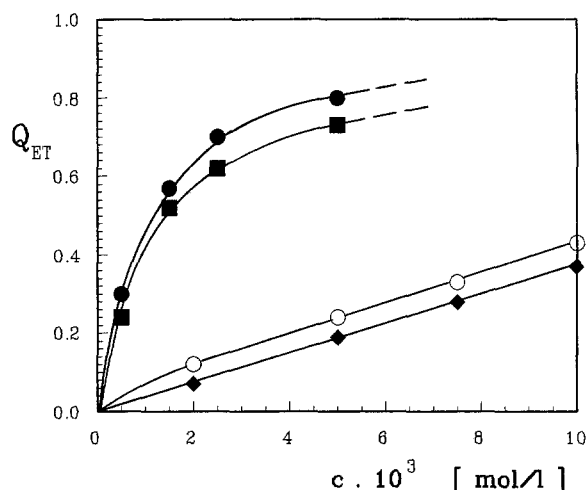
The efficiency of NET is

$$Q_{\text{ET}} = 1 - \left[ \frac{I_D(365)}{I_D^0(365)} \right]_{\text{corr.}}$$

where the indexes D and A refer to the donor and acceptor fluorophores, respectively,  $c$  is the molar concentration ( $M$ ), and  $\epsilon_\lambda$  is the molar absorption coefficient at the wavelength  $\lambda$  ( $\text{cm}^2 \text{mol}^{-1}$ ). The concentrations of the donor and acceptor fluorophores were the same in all measurements ( $c_D = c_A$ ) and all were carried out at 25°C.

## RESULTS AND DISCUSSION

The efficiencies of NET as a function of fluorophore concentration for copolymers **Ia:IIa** and **Ib:IIb** in methanol and water are shown in Fig. 1. Absorption and emission spectra of these copolymers were identical to that of low molecular weight analogues, and consequently intramolecular excimer formation does not take place (a low content of label). The fluorophores in copolymers are separated sufficiently so that energy migration is improbable. In view of the fact that the donor and acceptor are covalently bonded to the polymer carrier, molecular diffusion of the fluorophores during the



**Fig. 1.** Efficiency of NET,  $Q_{\text{ET}}$ , as a function of the fluorophore concentration for copolymers **Ia:IIa** in methanol ( $\circ$ ) and water ( $\blacklozenge$ ) and for copolymers **Ib:IIb** in methanol ( $\bullet$ ) and water ( $\blacksquare$ ).

donor excited-state lifetime is also very limited. Copolymer system **Ib:IIb** (a lower content of fluorophores) is much more efficient than system **Ia:IIa** (a higher content of fluorophores) from a  $Q_{\text{ET}}$  point of view; see Fig. 1. At the same molar concentrations of fluorophores the weight concentration of the copolymers in system **Ib:IIb** is two and half times higher than that in system **Ia:IIa**. At a molar fluorophore concentration  $c = 5 \times 10^{-3} M$ , it is 17 and 6.8 wt%, respectively. The increase in polymer concentration results in a viscosity increase in the measured solution. Although the fluorescence quantum yield of some compounds increases with increasing viscosity of the medium [12], this interpretation cannot completely explain the increase in  $Q_{\text{ET}}$  in system **Ib:IIb**. The second factor which probably has the main influence on NET is the concentration of polymers in the system. At a fluorophore concentration  $c = 5 \times 10^{-3} M$ , the

weight concentration of copolymers **Ia:IIa** (6.8 wt%) is comparable to the critical concentration of polymer coils,  $c_{\text{crit}}$  (wt%), determined according to  $c_{\text{crit}} = 2.5/[\eta]$  (for intrinsic viscosities  $[\eta]$ , see Table I) as  $c_{\text{crit}} = 4.5$  wt% for **Ia** and  $c_{\text{crit}} = 7.8$  wt% for **IIa**. If the copolymer concentration is lower than  $c_{\text{crit}}$ , the excluded volume effect is efficient and the polymer coils do not penetrate each other, and consequently the intermolecular donor-acceptor interactions responsible for NET are limited. If the copolymer concentration is higher (17 wt% for **Ib:IIb**) than the critical concentration ( $c_{\text{crit}} = 2.0$  wt% for **Ib** and  $c_{\text{crit}} = 6.0$  wt% for **IIb**), so according to Flory's theory the polymer coils penetrate one another. In this case intermolecular donor-acceptor interactions inside the polymer coils are also effective and the  $Q_{\text{ET}}$  increases.

## REFERENCES

1. T. Foerster (1959) *Discuss. Faraday Soc.* **27**, 7.
2. T. Foerster (1967) *Comprehens. Biochem.* **22**, 61.
3. I. Z. Steinberg (1971) *Annu. Rev. Biochem.* **40**, 83.
4. P. W. Schiller (1975) in R. F. Chen and H. Edelhoch (Eds.), *Biomedical Luminescence*, Marcel Dekker, New York, Vol. 1, Chap. 5.
5. N. J. Turro (1977) *Pure Appl. Chem.* **49**, 405.
6. V. Pokorná, F. Mikeš, J. Pecka, and D. Vyprachtický (1993) *Macromolecules* **26**, 2139.
7. F. Amrani, J. M. Hung, and H. Morawetz (1980) *Macromolecules* **13**, 649.
8. F. Mikeš, S. K. Dennis, and H. Morawetz (1980) *Macromolecules* **13**, 969.
9. F. C. Whitmore, H. S. Mosher, R. R. Adams, R. B. Taylor, E. C. Chapin, Ch. Weisel, and W. Yanko (1944) *J. Am. Chem. Soc.* **66**, 725.
10. H. L. Chen and H. Morawetz (1982) *Macromolecules* **15**, 1445.
11. J. Kopeček and H. Bažilová (1973) *Eur. Polym. J.* **9**, 7.
12. W. O. McClure and G. M. Edelman (1966) *Biochemistry* **5**, 1908.