

## Anthracene-Bound Fluorescence Studies of Methacrylic Acid-co-Methyl Methacrylate Copolymers

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A fluorescência de antraceno ligado a copolímeros de ácido metacrílico-co-metilmacrilato foi utilizada no estudo da conformação dos polímeros em solução aquosa. Copolímeros com diferentes proporções dos monômeros foram preparados e seu comportamento foi investigado em função do grau de ionização. Para os copolímeros mais hidrofóbicos a transição entre as conformações enovelada e estendida é deslocada para valores maiores de pH. Os resultados indicam que é necessária uma maior densidade de cargas para a expansão das cadeias. O microambiente para a sonda é discutido utilizando-se medidas dinâmicas de fluorescência. A análise dos decaimentos de fluorescência fornece duas distribuições de tempo de vida centradas ao redor 1.5 e 11.0 ns. Os decaimentos longos e curtos correspondem respectivamente à sonda colocada em uma esfera hidrofóbica e a microambientes polares permeáveis a água.

The conformation of copolymers of methyl methacrylate and methacrylic acid and the properties of its hydrophobic domains were studied using anthracene as a fluorescent probe bound to the polymer chain. Copolymers with different proportions of the monomers were prepared and their behaviour was investigated as a function of pH. The transitions between the coiled and the extended conformations of the copolymers occur at higher pH for the more hydrophobic copolymers, indicating that a larger amount of charges is necessary to expand these copolymers. A distribution of lifetimes is found for the copolymers, centered around 1.5 and 11 ns, the longer lifetime corresponds to the probe in the more hydrophobic microdomains, and this component remains even at high pH. The shorter lifetimes correspond to the probe in the aqueous environment.

**Keywords:** *polyelectrolytes, fluorescent probes, hydrophobic microenvironments*

### Introduction

The study of the photophysical and photochemical properties of polyelectrolytes in solution has become a field of growing interest due to the use of these compounds as new materials<sup>1</sup>. The possibility for these polymers to originate microheterogeneous domains when dissolved in water increased the interest in developing new systems. From an academic point of view, its importance may be associated with the ability of those systems to create unique microenvironments for reactions. They are also good models for some biological systems, rendering a variety of potential applications such as solar energy conversion, catalysis, drugs encapsulation etc<sup>2</sup>.

The presence of hydrophobic and hydrophilic groups on the macromolecular chain gives to these materials solubilization properties similar to those found for micellar

systems. The presence of a charged interface may accelerate or decrease the rate of reactions involving ions<sup>3</sup>.

All of these properties are directly related to the hydrophobicity, conformation and charge density on the chain, which are responsible for the behaviour of the system. Polyelectrolyte systems have been studied using various techniques, like rheological methods<sup>4</sup>, light scattering<sup>5</sup>, nmr<sup>6</sup>, as well as photophysical studies using fluorescent probes<sup>7</sup>.

Studies with fluorescent probes can be performed using three different approaches. The first one is the addition of the free probe, providing it has some characteristics which favour its incorporation to the microenvironments. This method has been used by us to detect the formation of aggregates in copolymers of vinyl acetate-co-sodium methallyl sulphonate<sup>8</sup>. The other methods are the binding of the probe to the polymer chain as a marker<sup>9</sup>, and the

inclusion of the probe to the macromolecular chain as a co-monomer in the polymerization process<sup>10,11,12</sup>. This last method eliminates any uncertainty about the incorporation of the probe to the system, or its adsorption to the desired sites, which may depend on external variables like temperature and pH. In the case of amphiphilic polyelectrolytes, the combination of hydrophobic and electrostatic interactions will affect the local microenvironment where the probe is placed. Therefore, the spectral or temporal response of the probe to conformational changes may provide relevant informations about the system. Furthermore, the knowledge of the behaviour of the probes in these media may be used to optimize photoinduced electron-transfer reactions<sup>13</sup>.

In this work, we want to report on results obtained from photophysical studies of anthracene bound to methacrylic acid-methyl methacrylate copolymers. The fluorescence of the anthracene moiety was monitored for different copolymers compositions and different charge densities on the chain, over a large pH range. The characteristics of the different microregions formed at various pH and charge density conditions are discussed in terms of the factors that maintain the chain in the compact conformation at low pH.

## Experimental

### Chemicals

9-Vinylanthracene (9-VA, Aldrich) was purified by crystallization from *n*-hexane. Methyl methacrylate (MMA, Aldrich) was washed twice with aqueous 5% NaOH and twice with water, dried with MgSO<sub>4</sub> and distilled twice at reduced pressure. Methacrylic acid (MAA) was dried with CaCl<sub>2</sub> and distilled twice under vacuum. Azobisisobutyronitrile, AIBN (Merck), was recrystallized from methanol.

### Synthesis of polymers

*Poly(methyl methacrylate-co-9-vinylanthracene)*. This polymer was prepared by copolymerization of both monomers using AIBN (0.5 % in mass) as initiator. 1.43 g (0.007 moles) of 9 VA were dissolved in 34.3 g (0.34 moles) of MMA, degassed by 4 thaw-and-freeze cycles and polymerized at 50 °C for 5 h. The polymer was dissolved in benzene and precipitated twice with hexane, filtered, vacuum-dried, and washed with hexane for 24 h in a soxhlet system.

*Poly(methyl methacrylate-co-methacrylic acid-co-9-vinylanthracene)*. The terpolymers were obtained by partial hydrolysis of the poly(MMA-9 VA) copolymer<sup>14</sup>. The hydrolysis was performed in dimethylsulphoxide, using NaOH 20%, under nitrogen and in the dark. The extent of the hydrolysis was regulated by controlling the temperature and the time of reaction. After the appropriate time, the reaction mixture was dilute with an equivalent volume of

water and the pH adjusted to 3.0 with concentrated HCl. The precipitate was washed with acidic methanol, precipitated with ethyl ether and vacuum-dried. The hydrolysis conditions used to obtain each copolymer are shown in Table 1.

*Poly(methacrylic acid-co-9-vinylanthracene)*. MAA (4.0 g, 0.046 moles) was copolymerized with 9 VA (0.2 g, 0.001 moles) in dimethylformamide, as described in the literature<sup>15</sup>.

**Characterization.** The molecular mass of the poly(MMA-9 VA) copolymer ( $M_w = 9.0 \times 10^4$ ,  $M_n = 5.1 \times 10^4$ ) was determined by GPC in THF on a Waters ALC/GPC-401 chromatograph with UV detection and poly(styrene-divinylbenzene) columns (Polymer Laboratories Plgel 10<sup>4</sup> Å + 10<sup>3</sup> Å + 500 Å) using polystyrene standards (100-500 000). The molecular mass of the poly(MAA-9 VA) copolymer ( $M_v = 8.0 \times 10^4$ ) was evaluated by viscosimetry, using the Mark-Howik constants for methanol<sup>16</sup>.

The amount of 9-VA in the copolymers was determined from the absorption spectra in methanol and acetone, using for the bound anthracene the same extinction coefficients of 9-methylanthracene, 9MA ( $\epsilon_{365} = 9230$  and  $9460 \text{ M}^{-1} \text{ cm}^{-1}$  in acetone and ethanol, respectively). These percentages were 0.120% and 0.065% in poly(methyl methacrylate-co-9-vinylanthracene) and poly(acrylic acid - 9-vinylanthracene), respectively.

The amount of MAA monomer (free acid) in the copolymers was determined by conductometric titration with NaOH in methanol/water (5:95 v:v). The properties of the copolymers are shown in Table 1.

### Measurements

All polymers were prepared from stock solutions in Milli-Q deionized water. The pH of the solutions was adjusted with concentrated HCl or NaOH and measured with a Micronal mod. B374 pH-meter, after letting the solution reach equilibrium. Titration of the polyelectrolytes ( $7-9 \times 10^{-4}$  equiv./L of methacrylic acid units) was performed with NaOH 0.01 M under N<sub>2</sub> atmosphere.

Fluorescence spectra were taken on Edinburgh Instruments CD-900 or Aminco-Bowman J4-896 spectrofluorimeters and absorption spectra were measured on an Hitachi U-2000 spectrophotometer. Fluorescence decay measurements were made by single photon counting with

**Table 1.** Hydrolysis conditions and composition of the copolymers.

Copolymer	COP1	COP2	COP3	COP4*
Reaction time (min)	120	120	180	—
Temperature (°C)	90	110	130	—
MAA (mol fraction)	0.39	0.67	0.87	~1.0
9VA (mol %)	0.12	0.12	0.12	0.065

a CD-900 Edinburgh Instruments spectrometer. The samples were excited at 370 nm, and the emission was measured at wavelengths larger or equal to 418 nm. The decays were evaluated with a bi-exponential function using the software provided by Edinburgh Instr. The same software was used to analyze lifetimes distributions.

## Results and Discussion

Figure 1 shows the absorption and fluorescence spectra of COP2 at different pH values. At low pH the quantum yield of the copolymers is about twice that observed at high pH. It is well known that the acid form of poly(acrylic acid) has a globular conformation, which expands when the pH of the solution is increased<sup>17</sup>. This expansion is due to the electrostatic repulsion between the charged carboxylate groups. The larger fluorescence quantum yield at low pH indicates that the bound anthracene is initially localized in an hydrophobic environment. The expansion of the chain exposes the fluorophore to a more polar microenvironment, in which the fluorescence quantum yield is lower.

The fluorescence intensity as a function of pH for all copolymers is shown in Fig. 2. All the systems present similar behaviour. There is an initial increase of the fluorescence intensity after a moderate increase in pH. At higher pH, this intensity falls abruptly to about 65-50 % of the initial intensity. It can be noticed that the decrease in fluorescence intensity observed for the more hydrophobic copolymers (COP1 and COP2) is less than for COP3 and COP4. This may be due to the latter having initially a less compact structure which allows the anthracene moiety to be in a more polar medium. The initial increase in the fluorescence quantum yield observed at low pH seems to be due to heterogeneous microenvironments formed by the interaction of the -COOH and -CH<sub>3</sub> groups of the hydrocarbon chain with the bound anthracene. A similar effect has already been observed for pyrene bound to poly(MAA)<sup>18</sup>. It can also be observed from Fig. 2 that the

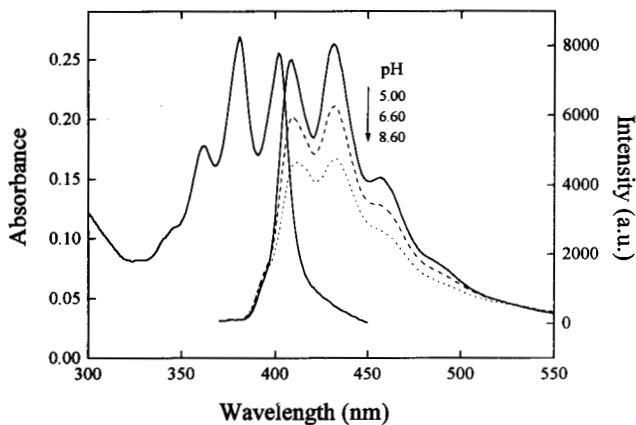


Figure 1. Fluorescence and absorption spectra of COP2 at (—) pH 5.0; (---) pH 6.6; and (· · ·) pH 8.6.

conformational transition of the more hydrophobic copolymers occurs at higher pH.

From pyrene fluorescence studies<sup>19</sup>, potentiometric titration<sup>20</sup>, and Raman spectrometry studies<sup>21</sup> it was found that the conformational transition of poly(MAA) happens between pH 5 and 6.5 ( $0.20 < \alpha < 0.30$ ). A similar result is found from anthracene fluorescence studies (Fig. 2). The displacement of the intensities curves for the more hydrophobic copolymers towards higher pH depends on the extent of the hydrophobic and hydrogen binding forces compared to the electrostatic forces. The pH of the conformational transitions of the copolymers can be compared considering that the mid-point of the conformational transition is where the initial intensity falls to 50%. This evaluation can be done calculating the average charge density at that point. The charge density parameter for a polyelectrolyte,  $\xi$ , is defined as<sup>22</sup>

$$\xi = \frac{e^2}{\epsilon \kappa T b} \quad (1)$$

where  $e$  is the electronic charge,  $\epsilon$  is the dielectric constant of the medium,  $\kappa$  is Boltzmann's constant,  $T$  is the temperature and  $b$  is the average distance between charges. For a totally ionized vinyl polymer  $b \approx 2.55 \text{ \AA}$ . At the mid-point of the transition the chains present more expanded conformations, so that the polyions may reasonably be approximated, at least locally, to a rigid cylinder. Assuming that the charged units of the copolymer are randomly distributed,  $b$  was calculated considering the composition of the copolymers<sup>23</sup> and the dissociation degree at the transition mid-point. The  $b$  values can be calculated from

$$b = 2.55/\alpha f \quad (2)$$

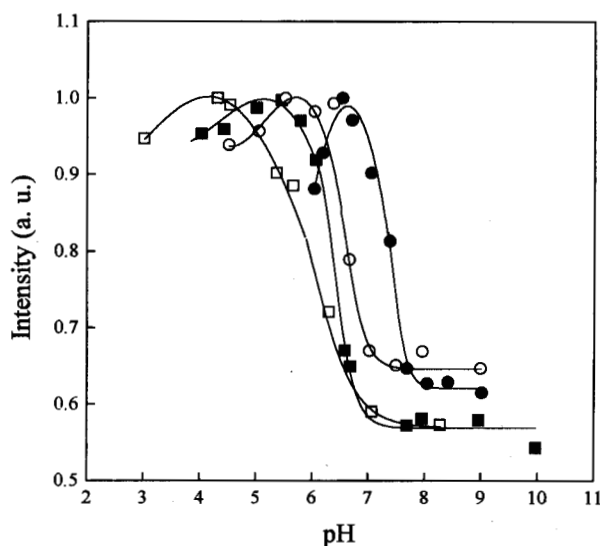


Figure 2. Fluorescence intensity as a function of pH for COP1 (●); COP2 (○); COP3 (■); and COP4 (□).

where  $f$  is the molar fraction of MAA in the copolymer. The dissociation degrees were taken from Fig. 3. The results of the titration of the copolymers with NaOH are shown in this figure as apparent "pK<sub>a</sub>" dissociation constants in function of the dissociation degree, with

$$\text{pK}_a = \text{pH} + \log [(1-\alpha)/\alpha] \quad (3)$$

The curves shown in Fig. 3 are similar to those obtained by Mandel and Stadhoudher,<sup>17</sup> and present non-monotone changes for low ionization degrees. These variations are emphasized for the more hydrophobic copolymers.

Charge densities are calculated at the conformational transition mid-point and the results are shown in Table 2. The calculated charge densities are always less than 1, which according to Manning<sup>22</sup> means that all the counterions are dissociated. Therefore, for highly hydrophobic copolymers, larger charge densities are required to expand the chain. As a consequence, the compact conformation of the polymers will be favoured at lower degrees of hydrolysis.

The factors that stabilize the compact form of the polymers have been discussed for a long time<sup>16,17,20,21</sup>. Mainly, two factors have been considered important: hydrophobic

interactions and intramolecular hydrogen bonds between ionized and non-ionized carboxyl groups<sup>18,19</sup>. Recent studies using viscosimetric and potentiometric techniques point to the importance of hydrogen bonds in the stabilization of the compact conformations of poly(methacrylic acid)<sup>24</sup>. This has also been found in Raman spectroscopy studies in the solid state and solution<sup>21</sup>. The results shown in Fig. 2 and Table 3, prove that a larger compactation can be expected for the more hydrophobic copolymers.

On the other hand, when going from COP1 to COP2, the acidic hydrogen atoms are gradually being replaced by methyl groups, so that the possibility of intramolecular hydrogen bonds will decrease. Therefore, we believe that the influence of the hydrogen bonds to stabilize the compact conformation is rather small when compared with the hydrophobic interactions. A similar behaviour has been found for copolymers of maleic anhydride with alkyl-vinylethers, where the conformational transition is not detected for hydrophobic copolymers containing C<sub>10</sub>-alkyl chains on the backbone<sup>25</sup>.

Fluorescence lifetimes for anthracene in COP2 and COP4 were measured at pH below and above the conformational transition. Fig. 4 shows the lifetime distribution for the decay of the probe in COP4. At low pH, the distribution is centered around 11 ns, with a small contribution of lifetimes around 1.5 ns. As the pH is increased the contribution of shorter lifetimes (up to 1.7 ns) increases. Even at high pH (> 8), when the conformational transition is expected to be completed, a contribution of the large lifetimes is still present, although the contribution of the shorter lifetimes keeps on increasing.

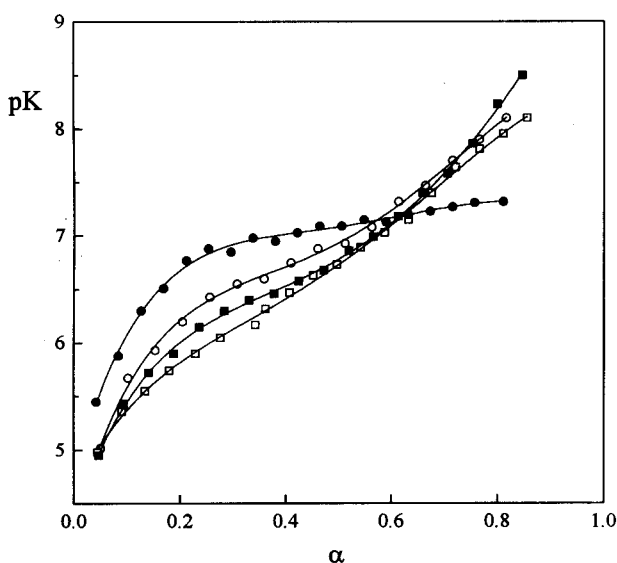
A statistical analysis allows an evaluation of the amplitudes corresponding to each group of lifetimes. The amplitude for the longer lifetime group (A<sub>1</sub>) is plotted in Fig. 5 together with the relative fluorescence intensities at the same pH. It can be concluded from Figs. 4 and 5, that the anthracene group will be localized basically in two different types of microenvironments during the process of disentanglement of the compact conformation. One corresponding to lifetimes around 11 ns, which can be

**Table 2.** Charge density at the conformational transition pH.

Copolymer	mid-point pH*	$\alpha$ †	b (Å)	$\xi$
COP1	7.37	0.87	7.54	0.97
COP2	6.68	0.39	9.83	0.72
COP3	6.27	0.29	10.28	0.69
COP4	5.97	0.24	10.71	0.67

\* From Figure 2.

† From Figure 3.



**Figure 3.** Change of pK with the degree of dissociation  $\alpha$  in water at 25°C for COP1 (●); COP2 (○); COP3 (■); and COP4 (□).

**Table 3.** Fluorescence Lifetimes of COP2 and COP4.

Polymer	pH	Lifetime (ns)		$\chi^2$
		$\tau_1$	$\tau_2$	
COP2	5.0	11.5	2.8	1.25
	7.0	11.0	2.8	1.14
COP4	4.0	11.5	2.5	1.25
	8.0	10.8	1.1	1.25
9-MeA (methanol)		4.3*		
9-MeA (hexane)		4.1*		

\* from Ref. 27.

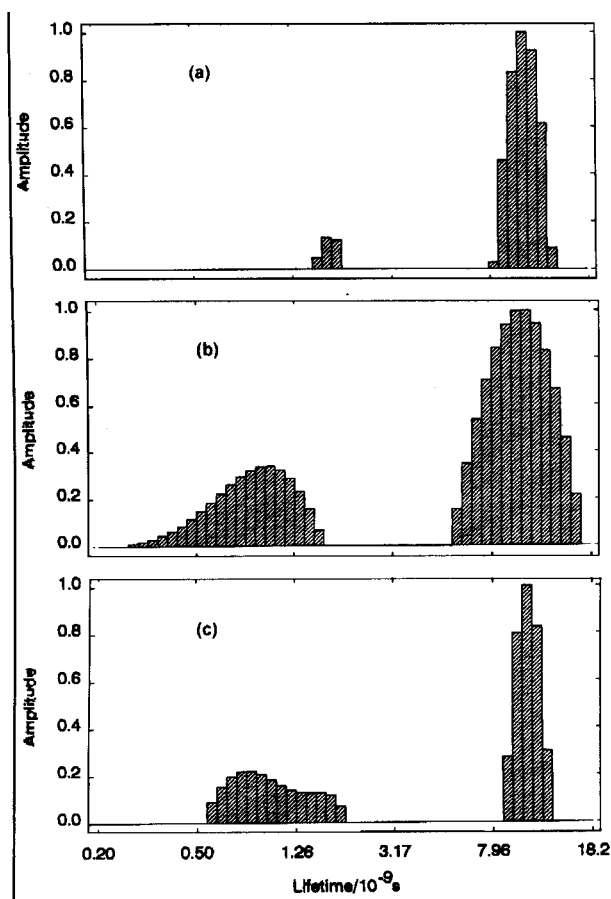


Figure 4. Lifetime distribution analysis for COP4 at (a) pH 4.5; (b) pH 6.5; and (c) pH 8.0.

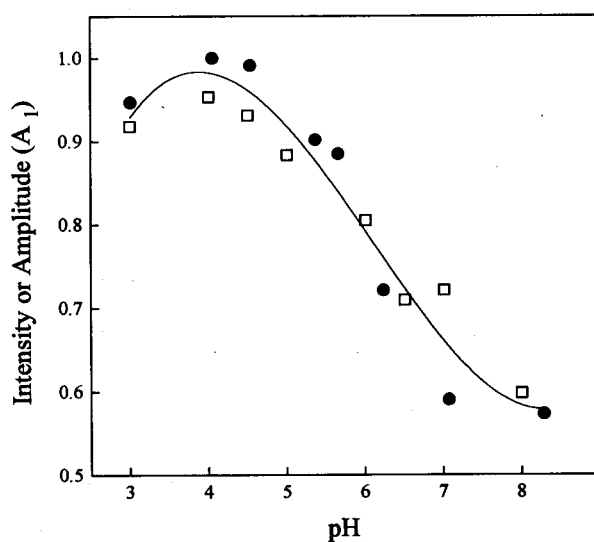


Figure 5. pH dependence for COP4 of the relative amplitude of the slow process from the lifetime distribution analysis  $A_1$  (□), and the relative fluorescence intensities (●).

assigned to the anthracene being in a quite hydrophobic, apolar and rigid medium, and a second one corresponding to a more aqueous environment with lifetimes up to 1.7 ns.

The decay data was also fitted with a bi-exponential function to obtain the lifetimes:

$$I(t) = B_1 \exp(t/\tau_{F1}) + B_2 \exp(t/\tau_{F2}) \quad (4)$$

A typical decay for COP2, and the fitting function is shown in Fig. 6. The fluorescence lifetimes of COP2 and COP4 at different pH are shown in Table 3, as well as the lifetimes of 9MA in different solvents. The lifetimes shown in that table are compatible with those obtained from the lifetimes distribution shown in Fig. 4. The lifetimes around 2 and 11 ns correspond to the probe being in polar and apolar microenvironments, respectively.

The longer decay time is about 2-3 times that of 9MA in methanol and hexane ( $\sim 4.3$  ns)<sup>26</sup>, indicating that the factor that lengthens the decay times is not only the polarity or hydrophobicity of the medium. The rigidity of the microenvironment, which precludes the possibility of internal conversion or other processes involving internal motions of the probe, will also contribute to extend the decay time.

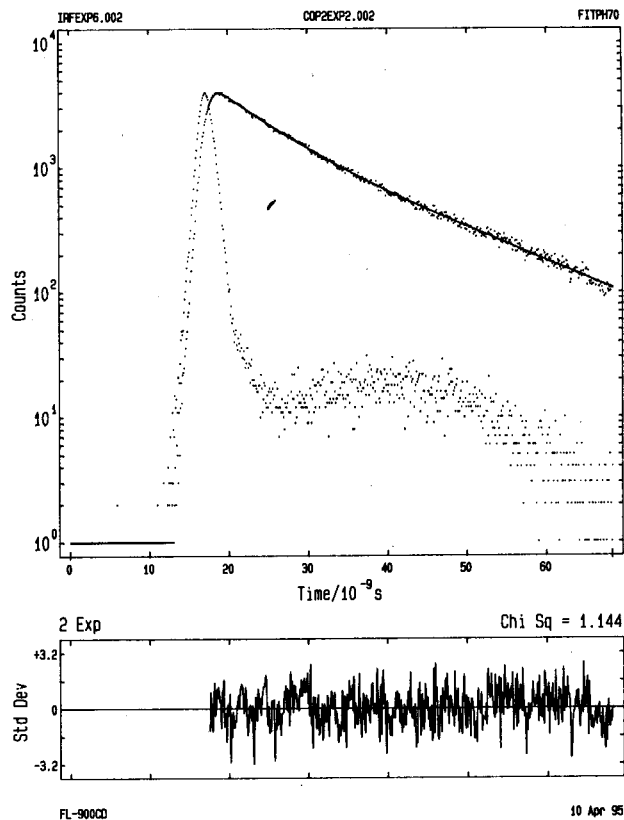


Figure 6. Fluorescence decay of anthracene bound to COP2 at pH 8.0. Dotted curves represent experimental points and the solid line represents the bi-exponential fitted curve.

The shorter decay time here reported should correspond to the probe in an aqueous environment. Due to low concentration of the anthracene moieties along the chain it quite difficult to assigned the short lifetime to an excimer. The quenching by oxygen can also be eliminated as extremely high concentrations (~0.1 M.) would be necessary for the process to be effective. Similar short lifetimes have been obtained recently by Tan<sup>26</sup> and Itoh<sup>27</sup> for anthracene bound to poly(MAA). Bednár *et al.*<sup>28</sup> found a similar behavior comparing dansyl in aqueous solution and dansyl labeled poly(MAA), for which the lifetimes fall from 22 to 6 ns. These authors interpreted the results assuming the existence of two distribution of probes. Therefore, the shorter decay time for anthracene will probably represent the population of the fluorophore placed in an aqueous microenvironment.

From the results obtained from multiexponential fittings of the decay curves, as well as from the lifetime distribution, it can be noticed that even for the presumably totally extended chain (at pH 8) emission corresponding to the longer lifetime is still present. Different microenvironments of anthracene bound to PMA at high pH were also found by Morisson *et al.*<sup>29</sup>

Therefore, the existence of hydrophobic microenvironment can be postulated even at higher pH values. These microregions can possibly be induced to some extent by the probe itself.

## Conclusions

The conformation of poly(MMA) and its copolymers depends on the hydrophobicity and charge density on the chains. Hydrophobic interactions are mostly responsible for the stabilization of the compact form of the polymers. Intramolecular hydrogen bonds seem not to be so important.

The anthracene moiety bound to the methacrylic acid copolymers presents a behaviour quite different from that in homogeneous solution. At low pH, the lifetimes are quite larger than those in organic solvents, indicating a more rigid environment for the probe.

The conformational transitions of the copolymers were determined by fluorescence measurements. For the more hydrophobic copolymers the chains are more compacted and the transitions occur at higher pH. Thus, higher charge densities will be necessary to expand the polymers.

Multiexponential decays are observed at all pH values, which is a prove that hydrophobic microdomains still exist at higher pH where the macromolecular chain is basically extended.

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