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Article

Time Resolved Fluorescence Anisotropy of Basic Dyes Bound to Poly(methacrylic acid) in Solution

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Soluções de poli(ácido metacrílico) atático, PAMA com peso molecular na faixa de (1,6 a 3,4) x 10^5 g mol^{-1}, e com corantes fluorescentes 9-aminoacridina ou azul do nilo ligados à cadeia, foram estudados por medidas fotofísicas em função da viscosidade e polaridade do solvente. O comportamento dos segmentos da cadeia do PAMA nas vizinhanças da sonda fluorescente foi descrito pela mudança na rotação difusional dos corantes. O etilenoglicol expande a cadeia polimérica quando comparada com a forma mais compactada do corante-PAMA em 50% água/etilenoglicol. A variação do tempo de relaxação rotacional do corante ligado ao PAMA indica uma expansão progressiva da cadeia polimérica para uma forma aberta em etilenoglicol.

Solutions of atactic poly(methacrylic acid), PMAA, with molecular weights in the range of (1.6 to 3.4) x 10^5 g mol^{-1}, and labeled with the fluorescent dyes 9-aminoacridine or Nile blue were studied by photophysical measurements as a function of solvent viscosity and polarity. The conformational behavior of the PMAA chain segments around the fluorescent probe was reported by the change in the rotational diffusion of the dyes. Ethylene glycol swells the polymer chain compared with the more contracted conformation of PMAA in 50% water/ethylene glycol. The change in the rotational relaxation time of the dye bound to PMAA with the decrease of water content in the solvent mixture indicates a progressive expansion of polymer chain to a more open coil form in solution.

Keywords*:* dyes, polyelectrolytes, PMAA, rotational diffusion, solvent friction

Introduction

Photophysical studies of polymers in solution have been extensively reported in recent years.[1,2] Time resolved fluorescence spectroscopy (TRFS) was found to be particularly useful in the study of several types of polymers in solution, and in the presence of additives.[3-10] Time resolved anisotropy decay of a fluorescent probe attached to a polymer chain reflects the rotational relaxation of the probe, and the local dynamics of the polymer segments in solution.[2]

The dependence of the segmental dynamics as a function solvent viscosity was studied by TRFS using anthracene attached to 1,2 polybutadiene.[11,12] The polymer local dynamics as well as the structural properties and relaxation processes of polymer and blends were studied by steady state and dynamic fluorescence using anthranyl and pyrenyl groups.[13-15] Fluorescence studies of polymethacrylic acid (PMAA) and polyacrylic acid (PAA) containing aromatic fluorescent probes such as naphthalene, anthracene and pyrene have been carried out by many authors to elucidate the properties of those polyelectrolytes in different solvents, pH, and surfactant addition.[16-20] Fluorescence anisotropy reflects the microviscosity and the solubilization dynamics of the probe molecule in solutions of polyelectrolytes. Reports in the literature using polarization and anisotropy of rhodamine 6 G in ethylene glycol demonstrate that the depolarization properties of the probe have relations with the solvent molecular alignment in jet flows.[21-22] Advanced procedures in data treatment, such as the global analysis method of the fluorescence anisotropy decay surface, have allowed the study of the rotation relaxation dynamics of fluorescent molecules in isotropic solvents and in non-homogeneous media.[23, 24]

The photophysical properties of thionine and phenosafranine dyes covalently bound to poly(acrylamidoglycol acid) and poly(methyloacrylamide) were investigated using stationary and TRFS measurements.[25] Although there are several reports of fluorescence anisotropy studies of

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aromatic probes in the presence of polymers, the rotational relaxation of a dye free in solution, and chemically bound to a macromolecule, has not yet been compared. The effects of the induced friction by the polymer chain in the rotational relaxation dynamics of the same dye probe in different situations, and its relation with the polymer conformation, are unknown. These questions are very important issues to several photophysical and photochemical studies of polymers in solution, including the energy transfer by the Förster mechanism.[26,27] The regime of dipole-dipole orientation in energy transfer is dictated by the rotational relaxation time of donor and acceptor molecules in the medium. Polymers labelled with fluorescent dyes are also currently studied by confocal and near-field optical microscopy, and by single-molecule fluorescence spectroscopy.[28] In addition, polymer with dyes covalently bound find potential application in molecular designing of photochemical sensors,[29] as well as in photodynamic therapy to sensitize singlet oxygen generation.[30]

In the present work, the photophysical properties of the dyes 9-aminoacridine and Nile blue bound to poly (methacrylic acid) are described. The effect of the viscosity in photophysical properties of these dyes is discussed in terms of changes in conformational properties of the polymer in solution. The use of mixtures of ethylene glycol and water is due to the fact that there are no reports studying the conformation of PMAA as a function of solvent viscosity.

Experimental

The dyes, 9-aminoacridine (Aldrich) and Nile blue (Aldrich) were recrystallized from methanol. Pyrene (Aldrich) was recrystallized from ethanol. Methacrylic acid (Aldrich) was distilled at low pressure prior to polymerization. 1,3-diisopropylcarbodiimida (Aldrich), N-methylmorpholine (Aldrich), were of analytical grade. All solvents used were of chromatographic grade (Mallinckrodt).

Electronic absorption spectra were measured with a Hitachi U-2000 spectrophotometer, and the corrected steady-state emission spectra were recorded using a CD-900 Edinburgh spectrofluorimeter. Fluorescence quantum yields (Φ_{em}) were calculated using quinine sulfate in 1 mol L^{-1} H_2SO_4 ($\Phi_{em} = 0.546$) and cresyl violet in methanol ($\Phi_{em} = 0.546$) standards,[31] in the case of acridine and Nile blue, respectively.

Fluorescence decays of the 9-aminoacridine (9AA) excited at 400 nm were measured by time correlated single-photon counting technique using a CD-900 Edinburgh spectrometer equipped with Glan-Thompson polarizers and a cooled PMT Hamamatsu R 955. The light pulse was provided by frequency doubling the 200 fs laser pulse of Mira 900 Ti – Sapphire laser pumped by a Verdi 5 W Coherent. The laser pulsed frequency was reduced by using a Conoptics pulse picker system. The fluorescence decays of Nile blue (NB) were measured in the same spectrometer but the excitation light was a 100 ps pulse of a Diode Laser system at 633 nm (Hamamatsu PLP 01). The fluorescence decays were analyzed by reconvolution of the instrument response function with exponential and anisotropy models of the Edinburgh Instruments software.

The rotational relaxation time τ_r was obtained by global fitting of the crossed and parallel polarization time resolved data using respectively,[32]

$$I_{\perp}(t) = \exp[-t/\tau_0]\left(1 - r_0 \exp[-t/\tau_r]\right) \quad (1)$$

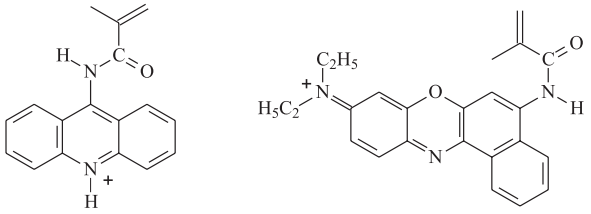
$$I_{\parallel}(t) = \exp[-t/\tau_0]\left(1 + 2r_0 \exp[-t/\tau_r]\right) \quad (2)$$

In the above equations, r_0 is the initial value of anisotropy, and τ_0 the fluorescence lifetime of the probe, which is obtained in a separated experiment measured at magic angle (54.7° from vertical polarizer) configuration and analyzed by single exponential fit.[32]

The molecular weights (M_w) of the polymers were determined by laser light scattering measurements using a Brookhaven Instruments BI 9000 AT equipped with a frequency doubled Nd-YAG laser (Uniphase, 125 mW at 532 nm). The M_w and the radius of gyration were calculated from the Zimm plots using the equipment data analysis software. The dn/dc values were determined in a Brookhaven Instruments (WGE Dr. Bures GmbH & Co. KG BI-DNDC) refractive index apparatus.

Synthesis of the dye labeled polymers

The first part consisted on the preparation of the dye-N-methacrylamide from the coupling reaction of the dye with MAA using 1,3-diisopropylcarbodiimide under nitrogen atmosphere and in the presence of a small amount of N-methylmorpholine following the procedure recently described in the literature.[33] The products were recrystallized from acetone, dried under vacuum, and characterized from ^1H and ^{13}C NMR, and FTIR spectral data. The molecular structures of the dye-N-methacrylamides are shown in Scheme 1. The poly(methacrylic acid), PMAA, labeled with the fluorescent dyes was prepared by free radical polymerization of MAA in the presence of a small amount of the dye-N-methacrylamide (0.3%), using benzoyl peroxide as thermal initiator under N_2 atmosphere and at 60 °C. The products were precipitated, centrifuged,



Acridine-9-N- methacrylamide Nile Blue-5-N-methacrylamide

Scheme 1. Molecular structures of the dyes-N-methacrylamide.

and washed several times with acetone to remove the non-reacting dye.

Results and Discussion

Polymer properties

The molecular weights, radius of gyration, and the average number of dyes per chain of the PMAA samples are reported in Table 1. The lower radius of gyration of the labeled polymers when compared with value of pure PMAA is ascribed to an electrostatic ion paring of the cationic dyes with dissociated acid groups that compacts the polymer coil in solution. The amount of dye was determined from the UV-visible spectral intensity assuming for the molar extinction coefficient the value of the free dye in the same solvent.[34-37] The electronic absorption and emission spectra of the dye-PMAA are illustrated in Figure 1. In the absorption measurements with dilute solution, no evidences of dimerization of the dye were observed in the spectra, indicating that the fluorescent probe is dispersed in the macromolecular media. In an ideal distribution of the dye, the probability of finding more than one dye in the same macromolecule, assuming the average close to 0.5, is less than 10%.

The tacticity of the PMAA was determined by ^{13}C and ^{1}H NMR measurements in D_2O solutions. The stereo-chemical splitting of the carbonyl and quaternary carbon signals (183.6, 184.4 ppm (-COOH) and 47.3, 47.7 ppm (-C(α)-)), together with the ^{1}H NMR signal of the CH_3 (broad band at 0.98 ppm which shifts to low field and splits into two peaks with temperature), are in agreement with the spectroscopic behavior of the reported data for atactic PMAA.[38,39]

Table 1. Molecular weight (M_w), gyration radius (R_g), and average number of dyes per polymer chain (⟨n⟩) of PMAA samples

Compound	M_w/(g mol^{-1})	R_g/(nm)	⟨n⟩
PMAA	194000	29.5 ± 1.2	
NB-PMAA	335000	19.4 ± 1.4	0.4
9AA-PMAA	162000	19.9 ± 1.7	0.5

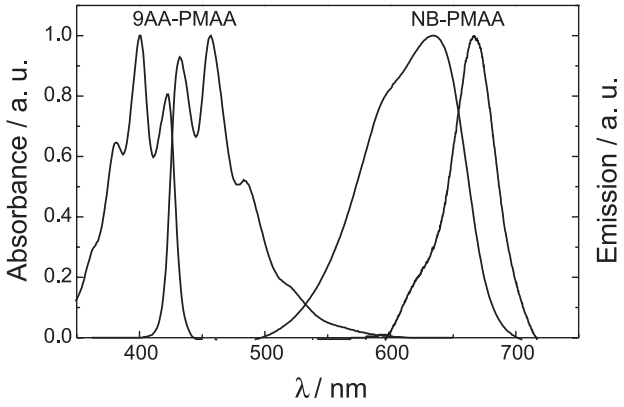


Figure 1. The electronic absorption and emission spectra of dye-PMAA in aqueous solutions.

Fluorescence lifetimes and quantum yields

The photophysical parameters of the excited singlet state of the dyes free in solution, and bound to PMAA are reported in Table 2. The lifetime of the acridine dye is practically constant in the solvents used. However, in the case of samples with NB, both lifetime and quantum yield change drastically with solvent. In particular, the small value of τ_0 (0.76 ns) and very low Φ_{em} (0.01) of free NB in water contrast with the larger values (τ_0 = 3.3 ns and Φ_{em} = 0.30) of the NB-PMAA in the same solvent. The change of lifetime and therefore of quantum yield of NB with solvent is ascribed to a strong effect of the solvent in the deactivation of S_1 excited state of the dye. In free NB in aqueous solution, nonradiative decay occurs also by partial charge-transfer of the NH_2 group leading to a change in the site of dye protonation. Therefore, the change in solvent polarity and viscosity as well as the presence of an amide group when linked to the polymer chain attenuate the TICT process, resulting in longer lifetime and higher quantum yield of the dye.

Table 2. Fluorescence quantum yield (ϕ_f) and lifetime (τ_0) of the dyes 9AA and NB in different solvents, adsorbed (dye/PMAA), and bound to polymer (dye-PMAA). [PMAA] = 2.6x10^{-5} mol L^{-1}, T = 298 K

Solvent	9AA		NB	
	ϕ_f	τ_0 / ns	ϕ_f	τ_0 / ns
methanol	0.45	14.6	0.16	4.79
ethanol	0.45	13.9	0.19	1.99
ethylene glycol	0.47	14.1	0.21	1.85
water	0.69	16.3	0.01	0.76
dye/PMAA (water)	0.86	16.2	0.27	3.12
dye-PMAA (water)	0.62	15.6	0.30	3.31

Fluorescence anisotropy

The fluorescence anisotropy results of the dye free in solution, and bound to PMAA, as a function of solvent viscosity are listed in Tables 3 and 4. The rotational relaxation times (τ_r) of the dyes observed in those systems are in the range of 100 ps up to a few ns. This indicates that the depolarization occurs mainly by local rotational diffusion of the dye in the solvent or in the polymer-solvent site. The model of exponential relaxation of the anisotropy of the dyes in these complex liquids is an approximation, and therefore τ_r should be considered an average value of the rotational relaxation motions of the probe in the solvent-polymer environments. Assuming that the cationic dyes free in a polar solvents are modeled as oblate ellipsoids under sticky condition, then:[32,40]

$$\tau_r = \frac{\eta V_h}{kT} \quad (3)$$

The behavior of the rotational relaxation time of the dyes as a function of solvent viscosity in dye free and dye-PMAA systems is illustrated in Figure 2. The τ_r of the free dye correlates linearly with the solvent viscosity according to the model given by equation 3, particularly in the region of low to moderate viscosity (η < 10 cP). However, when

Table 3. Fluorescence lifetime (τ_0), rotational relaxation time (τ_r) and the initial anisotropy (r_0) values of 9AA free, and bound to PMAA in different solvents, [dye-PMAA] = $3.1x10^{-5}$ mol L^{-1}, T = 298 K

Probe	Solvent	η / cP	τ_0 / ns	τ_r / ns	r_o / ns	χ^2
9AA	methanol	0.55	14.56±0.02	0.15±0.05	0.13±0.02	1.067
	water	0.89	16.28±0.02	0.22±0.10	0.13±0.02	1.011
	ethanol	1.20	13.92±0.02	0.22±0.09	0.14±0.02	1.033
	w/eg 80:20	1.45	15.24±0.02	0.17±0.08	0.19±0.01	1.033
	w/eg 60:40	2.50	14.64±0.03	0.18±0.08	0.22±0.01	1.009
	w/eg 40:60	4.05	15.05±0.03	0.21±0.06	0.24±0.01	1.056
	w/eg 20:80	8.00	14.4±0.03	0.36±0.04	0.26±0.02	1.098
	ethylene glycol	17.90	14.14±0.02	0.55±0.04	0.28±0.02	1.045
9AA-PMAA	methanol	0.55	13.95±0.02	0.20±0.08	0.13±0.02	1.024
	water	0.89	15.59±0.03	0.23±0.08	0.11±0.01	1.025
	ethanol	1.20	12.99±0.02	0.21±0.07	0.15±0.03	1.009
	w/eg 80:20	1.45	15.70±0.04	0.32±0.08	0.12±0.01	1.043
	w/eg 60:40	2.50	15.35±0.04	0.78±0.11	0.16±0.02	1.051
	w/eg 40:60	4.05	14.34±0.03	0.91±0.11	0.19±0.02	1.038
	w/eg 20:80	8.00	13.87±0.06	0.97±0.01	0.19±0.01	1.044
	ethylene glycol	17.90	13.18±0.03	0.99±0.06	0.21±0.02	1.041

w = water; eg = ethylene glycol. χ^2 is the reduced chi-square value of the anisotropy decay fitting.

Table 4. Fluorescence lifetime (τ_0), rotational relaxation time (τ_r) and the initial anisotropy (r_0) values of NB free, and bound to PMAA in different solvents, [dye-PMAA] = $1.5x10^{-5}$ mol L^{-1}, T = 298 K

Probe	Solvent	η / cP	τ_0 / ns	τ_r / ns	r_o / ns	χ^2
NB	Methanol	0.55	4.79±0.56	0.10±0.03	0.29±0.03	1.107
	Water	0.89	0.76±0.11	0.13±0.04	0.22±0.02	1.005
	Ethanol	1.20	1.99±0.33	0.26±0.02	0.26±0.02	1.183
	w/eg 80:20	1.45	2.04±0.21	0.11±0.02	0.20±0.02	0.991
	w/eg 60:40	2.50	1.04±0.12	0.12±0.04	0.17±0.02	0.984
	w/eg 40:60	4.05	1.27±0.42	0.25±0.05	0.29±0.02	1.029
	w/eg 20:80	8.00	2.31±0.09	0.34±0.05	0.35±0.01	1.047
	ethylene glycol	17.90	1.85±0.17	1.21±0.15	0.36±0.05	1.121
NB-PMAA	Methanol	0.55	1.58±0.23	0.24±0.09	0.34±0.06	1.119
	Water	0.89	3.31±0.32	0.40±0.19	0.22±0.02	1.034
	Ethanol	1.20	1.91±0.19	0.86±0.09	0.36±0.03	1.088
	w/eg 80:20	1.45	3.11±0.21	0.86±0.09	0.25±0.01	1.056
	w/eg 60:40	2.50	2.44±0.12	0.97±0.34	0.26±0.01	1.067
	w/eg 40:60	4.05	1.42±0.21	1.14±0.26	0.24±0.01	1.078
	w/eg 20:80	8.00	1.35±0.11	1.26±0.06	0.24±0.01	1.088
	ethylene glycol	17.90	1.26±0.22	1.75±0.26	0.21±0.02	1.079

w = water ; eg = ethylene glycol. χ^2 is the reduced chi-square value of the anisotropy decay fitting.

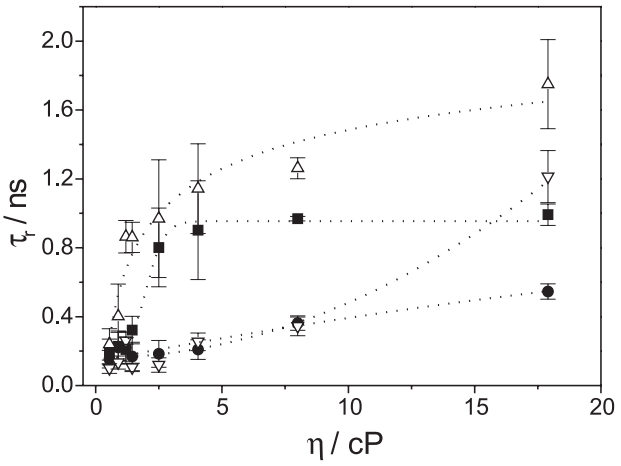


Figure 2. Rotational relaxation time (τ_r) values as a function of solvent viscosity for 9AA free (●), 9AA-PMAA (■), NB free (▽), and NB-PMAA (△). [dye-PMAA] = 3.1 and 1.5x10^{-5} mol L^{-1} for 9AA-PMAA and NB-PMAA respectively, T = 298 K.

the dye is bound to PMAA, its rotational diffusion becomes much slower than that of the free dye in the same solvent, and the larger difference is observed upon the first addition of fraction of ethylene glycol in water. The nonlinear behavior of the rotational relaxation time (τ_r) of NB at higher solvent viscosity can be ascribed to a clustering effect of tightly bound solvent molecules around the dye. In such case, V_h is not constant, but it increases due to strong solvent-solute interaction.

The higher friction felt by the dye in the polymer environment may be ascribed to two additional contributions. The first one arises from the coupling of segmental motion of the PMAA monomers surrounding the solvated dye with its rotational diffusion (hydrodynamic constraint). The rotational relaxation of the dye is partially hindered when it is bound to the polymer chain. The second is a dielectric friction contribution, which ultimately depends on the ion paring and hydrogen bonding of the dye with acid groups of the PMAA polymer and dielectric relaxation of the solvent. It is very difficult to separate these two contributions. But, assuming an effective friction or viscosity that takes into account all contributions over the same dye hydrodynamic volume under sticky conditions, the ratio of the rotational relaxation time of the dye bound to PMAA and free in solvent may be written approximately as,

$$\frac{\langle \tau_r \rangle_P}{\tau_r} = \frac{\langle \eta \rangle_P}{\eta}, \qquad (4)$$

where $\langle \eta \rangle_P$ is the effective friction or viscosity felt by the dye in the polymer environment. The plot of the experimental data according to equation 4 as a function of solvent viscosity is given in Figure 3. The maximum of the curves, for both dyes bound to PMAA, occurs in a similar region of viscosity, but with different amplitudes. It corresponds to a solvent viscosity of about 2.5 - 3.0 cP (mixture of 40 - 50% of water/ethylene glycol). The dielectric constant in this solvent composition is 65 at 293 K. The change in the polarity of the solvent that affects directly the dielectric friction contribution is not the main driving factor of the change observed. It seems that in a particular solvent composition there is a maximum folding of the polymer chain forming a compact or contracted coil in which the dye is embedded. This effect increases the local viscosity $\langle \eta \rangle_P$. However, further increase of the percentage of ethylene glycol in solution does not increase the viscous friction sensed by the probe, and its rotational relaxation time becomes constant, approaching the value found for the free dye. This behavior could be ascribed to a partial disruption of the hydrophobic aggregates of methyl groups of PMAA when increasing the amount of ethylene glycol in the solvent. This process gives an expansion corresponding to a swelling of the PMAA chain, and therefore exposes more the dye to the solvent environment.

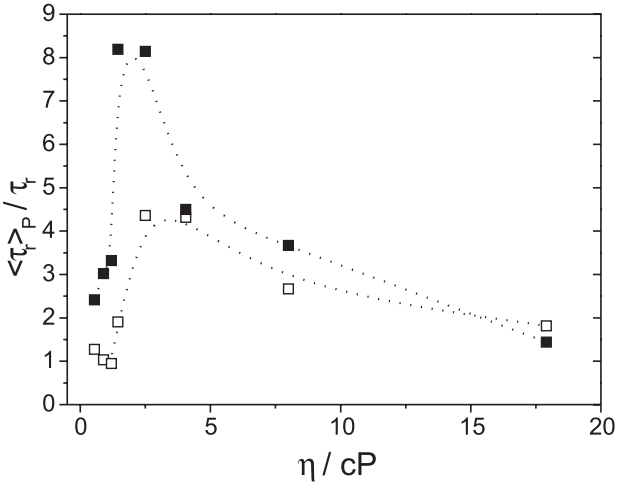


Figure 3. The ratio of the rotational relaxation times of dye-PMAA and dye free as a function of solvent viscosity in water/ethylene glycol mixtures. 9AA-PMAA (□) and NB-PMAA (■). [dye-PMAA] = 3.1 and 1.5x10^{-5} mol L^{-1} for 9AA-PMAA and NB-PMAA respectively, T = 298 K.

Conclusions

Solutions of PMAA labeled with fluorescent dyes acridine and Nile blue were prepared and the systems were studied in different solvents. The fluorescent probes used here are cationic dyes with good solubility in polar solvents and in water, contrary to the neutral aromatic probes extensively employed in fluorescence anisotropy studies of labeled polymers, which are highly hydrophobic compounds. Thus the dyes used here report predominantly the changes near the aqueous interface of the labeled

polymer in solution. The rotational relaxation of the dye, measured by time resolved fluorescence anisotropy, correlates with the structure of solvated polymer segments around the dye, and therefore reflects the local conformational changes of labeled PMAA in solution. Highly viscous and polar solvents like ethylene glycol show an effect of swelling of the polymer chain producing a coil expansion when compared with the more contracted conformation achieved by dye-PMAA in 50 % water/ethylene glycol. In general, solvent local ordering due a strong solvent-solvent interaction (hydrogen bonding in ethylene glycol for instance), which ultimately accounts for high viscous solvent behavior, has an important role on the local conformation of a long polymer chain in solution reported by fluorescence anisotropy measurements.

Acknowledgments

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