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BODIPY-Conjugated Thermo-Sensitive Fluorescent Polymers Based On 2-(2-methoxyethoxy)ethyl methacrylate

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ABSTRACT: A methacrylic monomer containing the dye 4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacene (BODIPYMA) was synthesized to provide fluorescence properties to three different thermo-sensitive families of polymers based on 2-(2-methoxyethoxy)ethyl methacrylate (MEO_2MA). Initially, linear random terpolymers of MEO_2MA, oligo(ethylene glycol) methyl ether methacrylate ($OEG_{8-9}MA$) with M_n = 475 g mol^{-1}, and a very low proportion of BODIPYMA were synthesized by atom transfer radical polymerization (ATRP). These terpolymers showed lower critical solution temperature (LCST), which values were easily tuned by changing the monomeric composition, and fluorescence, which intensity increases significantly after this thermal transition. Second, a family of diblock copolymers was formed by the ATRP of MEO_2MA and a very short block of BODIPYMA. They also behave as a fluorescent thermometer in water but, in this case, the fluorescence quantum yield decreased due to the intermolecular $\pi-\pi$ stacking of BODIPY dyes. In addition, a MEO_2MA-BODIPYMA based hydrogel was prepared to show that the optical and fluorescence properties also exhibit a sudden and reversible change at the volume transition temperature (VTT) of the hydrogel.

Introduction

In the last few years, the number of contributions where the 4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacene (BODIPY) dyes appear is exponentially increasing.[1,2] Recently, the BODIPY dyes have been used as optical sensitivity compounds for biochemical labeling,[3] solar cells,[4] or luminescent ionic liquid crystals.[5] These dyes present advantageous characteristics versus other chromophores, such as high extinction coefficients, high quantum yields, low triplet−triplet absorption and the fact that its excitation wavelength lies in the visible-wavelength range.[6,7]

The covalent incorporation of BODIPY dyes into a polymer main chain,[8−13] side chain,[14,15] or initial end[16] has drawn attention during the last years. In fact, our research group has demonstrated that novel BODIPY-based polymers prepared by the free radical copolymerization of methyl methacrylate (MMA) with methacrylic BODIPY monomers,[17,18] exhibited efficient and high photostability toward laser in liquid and in solid polymeric matrices. Besides, BODIPY cross-linker monomer has showed better results than the former.[19] Although BODIPY-based polymers are being investigated, just few articles report the use of thermo-sensitive polymers to obtain fluorescent temperature sensors.[20,21] On the one hand, Wang et al.[20] synthesized by free radical random copolymerization a system composed of *N*-isopropylacrylamide (*N*-iPAAm) and BODIPY monomer having a meso-pyridinium group. These polymers act as a fluorescence-enhancement-type thermometer and displayed reversible fluorescence enhancement/quenching at the lower critical solution temperature (LCST) of P(*N*-iPAAm), due to the inhibition of the rotation of the mesopyridinium group in the BODIPY after the polymer collapse.[20] On the other hand, Nagai et al.[21a] studied random copolymers based on 2-dimethylaminoethyl methacrylate (DMAEMA) and a BODIPY-based monomer synthesized by reversible addition−fragmentation chain transfer polymerization (RAFT). This is also a very interesting work in which the luminescent intensity of the obtained also depends on the temperature.

In the present contribution, we propose fluorescent thermo-sensitive polymers with controlled molecular weigh and structure, based on 2-(2-methoxyethoxy)ethyl methacrylate (MEO_2MA) with a very low proportion of BODIPY methacrylic monomer (BODIPYMA). This system is going to present reversible change in the fluorescence intensity with the temperature but also, some advantages that clearly improve the properties of the system in comparison to the previous works.[20,21a] Thus, MEO_2MA-based polymers present thermo-responsive behavior generally comparable to P(*N*-iPAAm),[28] showing a LCST at around 26 °C,[22] but they show the advantage that this transition temperature can be easily modulated by copolymerization of MEO_2MA with longer oligo(ethylene glycol) (OEG) methacrylates without losing the sharpness of the transition.[23−27] Another remarkable aspect in comparison with the previous compounds is that our system shows good biorelated properties (i.e., nontoxicity, anti-immunogenicity) since they are formed by oligo(ethylene glycol) side chains. In addition, they can be successfully obtained from controlled/living polymerizations techniques, such as atom transfer radical polymerization (ATRP),[23−25,29,30] that will allow a control over the molecular weight and the molecular weight distribution. Moreover, MEO_2MA has demonstrated to be a good monomer in order to synthesize thermo- and pH-sensitive hydrogels, which has been the focus of our more recently investigations.[31,32] Furthermore, the last novelty of this work is that the chromophore group in BODIPYMA is tethered to the methacrylic group by a short methylene chain which will allow a better coupling to the dynamics of the thermo-sensitive polymer, increasing the expected thermal fluorescent response.

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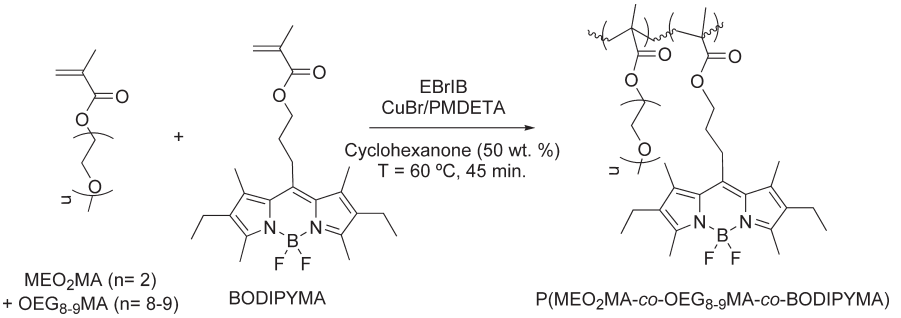
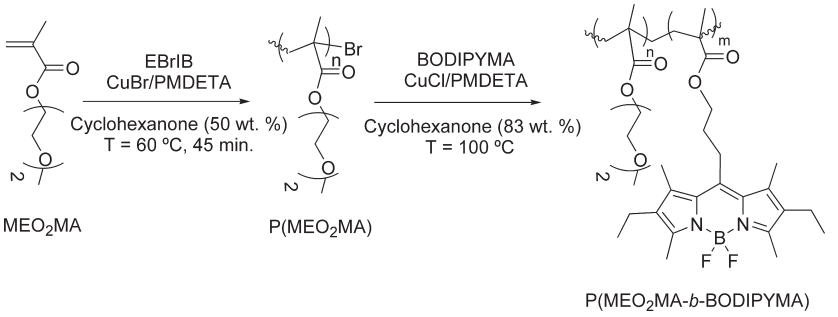
Scheme 1



Scheme 2



Taking all these facts into account, linear random terpolymers of MEO_2MA, oligo(ethylene glycol) methyl ether methacrylate ($OEG_{8-9}MA$) with M_n = 475 g mol^{-1} and a very low proportion of BODIPYMA were synthesized by ATRP, according with Scheme 1. The main aim of this strategy was to modulate the temperature at which the increase of the fluorescence occurs by tuning the LCST of the terpolymers, which can be easily done by changing the ratio of the two OEG monomers.[23−27] A second aim was to evaluate if the luminescent properties of these fluorescent thermometers in water depend on the $\pi-\pi$ stacking interactions originated from the planar structure of BODIPY. Thus, a two step ATRP synthesis (Scheme 2) was employed to obtain diblock copolymers. To the best of our knowledge this is the first example of the study of thermo-sensitive block copolymer based on BODIPY dyes. Thus, the fluorescent properties were studied as a function of the temperature and compared with the properties of the statistical copolymers. In addition, a BODIPY-based hydrogel, where the dye is covalent bound to a thermo-sensitive network, was synthesized for the first time to show that this relevant combination of thermo-sensitivity and luminescence can also be used in cross-linked structures. The reduction of void or free volume surrounding the dye at the volume transition temperature (VTT) is going to restrict the molecular motions giving rise to nonradiative decay, and thus enhances the emission intensity.

Experiments

Materials. The monomers 2-(2-methoxyethoxy)ethyl methacrylate (MEO_2MA, Aldrich 95%) and oligo(ethylene glycol) methyl ether methacrylate with M_n = 475 g mol^{-1} ($OEG_{8-9}MA$, Aldrich) were purified by passing by neutral alumina column to remove the inhibitor. The ligand *N*,*N*,*N*′,*N*″,*N*″-pentamethyldiethylenetriamine (PMDETA, Aldrich 99%) was purified by vacuum distillation. The initiator ethyl 2-bromoisobutyrate (EBrIB, Aldrich 99%), the catalyst precursors copper bromide (CuBr, Aldrich 99.999%) and copper chloride (CuCl, Aldrich 99.99%), the solvents cyclohexanone (Scharlau 99.5%), *n*-hexane (Panreac 98%), acetone (Carlo Ebra 99.8%), toluene (Merck 99.9%), dichloromethane (DCM, Merck 99.8%), ethanol (Normapur, analytical reagent) and chloroform (Sds 99.9%) were used as received. The BODIPY derivative 4,4-difluoro-1,3,5,7,8-pentamethyl-2,6-diethyl-4-bora-3a,4a-diaza-*s*-indacene (PM567, laser grade from Exciton, OH) used as reference in the measurement of the photophysical properties was also used without previous purification. The chemicals used for the synthesis of the monomer BODIPYMA[17] were purchased from Aldrich (Steinheim, Germany) and were used without further purification, except 3-ethyl-2,4-dimethylpyrrole, which was distilled.

For the synthesis of the hydrogel, the activator *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TEMED, Fluka 99%), the conventional cross-linker tetraethylene glycol dimethacrylate (TEGDMA, Fluka 90%) and the initiator ammonium peroxodisulfate (APS, Fluka 98%) were also used as received. Phosphate buffer solutions (PBS) were prepared employing water (Milli-Q) from water purification facility (Millipore Milli-U10), sodium dihydrogen phosphate anhydrous (Fluka ≥99%), disodium hydrogen phosphate (Panreac ≥98%), ortho-phosphoric acid (Panreac 85%), and sodium chloride (Panreac ≥99.5%) to keep constant the ionic strength (μ).

Synthesis of the Monomer BODIPYMA. The synthesis of BODIPYMA, a methacrylic monomer with a BODIPY dye (analogous to PM567) tethered by a spacer of three methylene groups, was made according with the procedure described previously by our research group.[17] Its structure can be observed in Scheme 1.

Syntheses of P(MEO_2MA-*co*-$OEG_{8-9}MA$-*co*-BODIPYMA) Terpolymers. Terpolymerizations were carried out by ATRP at 60 °C in cyclohexanone solution (50 wt %) with a constant monomer/initiator concentration ratio of 100:1 and with the monomeric compositions showed in Table 1, being in all cases $[MEO_2MA+OEG_{8-9}MA]_0/[BODIPYMA]_0$ = 1400:1. Amine ligand (PMDETA) (46 mg, 0.266 mmol), degassed monomers ($[MEO_2MA]_0+[OEG_{8-9}MA]_0$ = 26.6 mmol), BODIPYMA (8 mg, 0.019 mmol), CuBr (38 mg, 0.266 mmol), and solvent (previously bubbled with nitrogen for at least 15 min) were all added to dry Pyrex tube

Table 1. Experimental Synthetic Conditions, Polymer Characterization, and Photophysical Properties of P(MEO_2MA-*co*-$OEG_{8-9}MA$-*co*-BODIPYMA) Terpolymers[a]

								photophysical properties				
entry	f_{MEO2MA}	F_{MEO2MA}	conv (%)	$M_{n,theor.}$ (g mol^{-1})	$M_{n,SEC}$ (g mol^{-1})	M_w/M_n	cloud point (°C)	solvent	λ_{abs} (nm)	λ_{em} (nm)	ε (M^{-1} cm^{-1})	Φ_{flu}
T1	1.00	1.00	60.4	11 570	19 655	1.39	27.2	acetone	521	535	4900	0.86
								toluene	525	539	5600	0.70
								ethanol	522	535	5600	0.87
								DCM	524	536	2600	0.56
								PBS[b]	524	535	4950	0.67
T2	0.95	0.95_6	63.6	12 950	20 846	1.50	34.8	PBS[b]	524	535	4950	0.67
T3	0.85	0.87_1	59.7	13 640	24 476	1.57	46.1	PBS[b]	524	535	5900	0.67

[a] The photophysical properties were measured in 0.36 mg mL^{-1} solutions at 10 °C. [b] pH = 7, μ = 0.1 M.

Table 2. Experimental Synthetic Conditions, Polymer Characterization, and Photophysical Properties of P(MEO_2MA-*b*-BODIPYMA) Block Copolymers Synthesized by ATRP Using a P(MEO_2MA) Macroinitiator Previously Synthesized by ATRP (Conv = 72%, $M_{n,SEC}$ = 19686 g mol^{-1}, M_w/M_n = 1.41, LCST = 27.6 °C)

						photophysical properties in PBS (pH 7)			
entry	time (min)	$[M]_0/[I]_0$	$M_{n,SEC}$ (g mol^{-1})	M_w/M_n	cloud point (°C)	λ_{abs} (nm)	λ_{em} (nm)	ε (M^{-1} cm^{-1})	Φ_{flu}
B1	60	45	20 889	1.41	26.4	524	532	52 800	0.10
B2	20	10	20 423	1.42	27.8	524	534	26 300	0.08

ampules. Next, the polymerization mixtures were carefully degassed by bubbling nitrogen during 20 min, and then the initiator (EBrIB) (39 μL, 0.266 mmol) dissolved in cyclohexanone (2 mL) was introduced into the ampules using degassed syringes in order to start the polymerization. The ampules were immediately placed in a thermostatic oil bath, regulated with a precision of ±0.1 °C. When the desired time (45 min) was up, the reaction mixture was quenched opening the ampule and exposing the content to air, cooled in an ice/water bath and adding chloroform (30 mL). Then, the reaction product solution was passed through a neutral alumina column to remove the catalyst. The solution was concentrated by rotary-evaporation, and the polymer solution was poured into a large excess of *n*-hexane (600 mL). The precipitated products were decanted and dried until a constant weight was reached. Total monomer conversions were measured gravimetrically.

Syntheses of P(MEO_2MA-*b*-BODIPYMA) Copolymers. The synthesis of these diblock copolymers was made in two steps. Initially, a P(MEO_2MA) homopolymer was synthesized using the procedure described in the last section but without BODIPYMA. Then, it was used as macroinitiator for the polymerization of BODIPYMA in a second step. These chain extensions were carried at 100 °C, in cyclohexanone solution (1:5 wt./wt.) and with a constant macroinitiator/CuCl/PMDETA concentration ratio of 1:1:1. The synthetic procedure was the same previously described for the statistical terpolymers. The relationship between macroinitiator and BODIPYMA monomer and the time of reaction are detailed in Table 2.

Synthesis of P(MEO_2MA-*co*-BODIPYMA) Hydrogel. A P(MEO_2MA-*co*-BODIPYMA) hydrogel was synthesized by free radical cross-linking random polymerization in solution using $[MEO_2MA]_0/[BODIPYMA]_0$ = 1400:1. Polymerization was carried out in a mixture water/ethanol 1:1 (v/v) as solvent with TEGDMA. In all cases the monomers/solvent ratio was 1:1 (w/w). Cross-linker TEGDMA, activator TEMED, and initiator APS were used with an initial ratio of 0.5 wt % of the total monomer amount. The procedure to obtain the hydrogels in the desired shape is described elsewhere.[31]

Characterization. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker 300 MHz spectrometer in $CDCl_3$ at room temperature. Molecular weights (M_n) and molecular weight distributions (MWD) were determined by SEC with a GPC Perkin-Elmer (mobile phase: DMF with LiBr (0.1 wt %) at 0.3 mL min^{-1} and 70 °C) with a differential refractometer Waters 410 detector. Poly(methyl methacrylate) (PMMA) standards were employed for the calibration. The LCST was studied by determining the optical transmittance at 600 nm of the copolymers in PBS as a function of temperature. The analysis was made using a Cary 3 BIO-Varian UV−visible spectrophotometer. The temperature was raised from 5 to 80 °C at a rate of 1 °C min^{-1}. The LCST (cloud point) was defined as the temperature at the inflection point of the absorbance vs temperature curve. UV−vis absorption and fluorescence spectra were recorded on a Perkin−Elmer Lambda-16 and on a Perkin-Elmer LS5OB spectrophotometer, respectively. Fluorescence signals (excitation at 490 nm) were recorded in the front-face mode by orienting the sample at 35° and 55° with respect to the excitation and emission beams, respectively when the measures were done at different temperatures. The recorded fluorescence spectra were the average of at least three independent measurements made until reproducible fluorescence intensity was reached. The fluorescence quantum yield (Φ) (excitation at 490 nm) was evaluated relative to the PM567 dye in ethanol solution (Φ = 0.86).[33] The samples were thermostatized using a Huber-polycat cc1 cryostat system or a Julabo-paratherm U5-electronic thermostatized bath. For the hydrogel, the equilibrium swelling (Q_∞) was calculated in grams of solvent incorporated per grams of dry gel (xerogel) at 24 h of swelling.

Results and Discussion

P(MEO_2MA-*co*-$OEG_{8-9}MA$-*co*-BODIPYMA) Terpolymers. ATRP was used to synthesize random terpolymers based on MEO_2MA (Scheme 1), in which $OEG_{8-9}MA$ was used as comonomer to modulate the LCST,[22−25,34−40] and BODIPYMA was used in a constant and low proportion to produce luminescent compounds.[17,18] Experimental synthetic conditions and properties of the obtained copolymers are summarized in Table 1. It should be noted that, in all cases, a high feed molar ratio of MEO_2MA (f_{MEO2MA}) was used to obtain polymers with low LCST, since the LCSTs of P(MEO_2MA) and P($OEG_{8-9}MA$) are of ∼26 and ∼90 °C, respectively.[23]

The chemical structure of the obtained polymers was analyzed by ^{1}H NMR (Figure S1 in the Supporting Information),

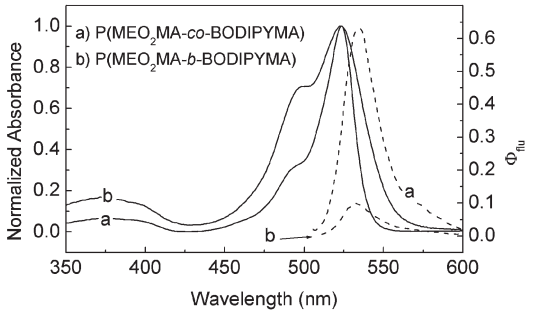


Figure 1. UV−vis normalized absorption (solid lines) and fluorescence spectra (dashed lines) of (a) P(MEO_2MA-*co*-BODIPYMA) copolymer (sample **T1**) and (b) P(MEO_2MA-*b*-BODIPYMA) diblock copolymer (sample **B1**) both dissolved in PBS (pH 7, μ = 0.1 M) at 10 °C.

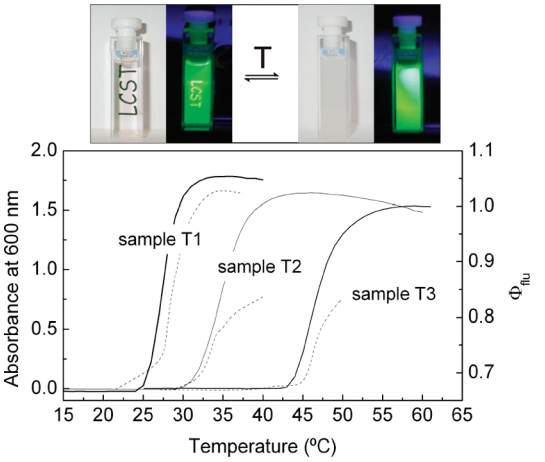


Figure 2. Absorbance at 600 nm (solid lines) and fluorescence quantum yield (dashed lines) vs temperature of the P(MEO_2MA-*co*-OEG_{8-9}MA-*co*-BODIPYMA) terpolymers dissolved (0.36 mg mL^{-1}) in PBS (pH 7, μ = 0.1 M). Photographs under visible and UV irradiation taken at temperatures below LCST (left part) and above LCST (right part) of sample **T1** are included.

mainly observing those proton signals of the oligo(ethylene glycol) macromonomer units.[25] Thus, the experimental molar ratios of MEO_2MA in the copolymer (F_{MEO2MA}), which values are shown in Table 1, could be estimated from the ratio between the intensity of the signal corresponding to the protons of the central OCH_2 groups and the intensity of the rest of signals. These experimental values are quite close to those used in the feed. This, together with the fact that conversions achieved are quite similar for all monomeric compositions, indicates that the two main monomers present similar reactivity ratios under these conditions, as it could be expected from their similar chemical structure. This is also a relevant result since it allows to easily obtain the desired composition in the polymers. The SEC analysis of the copolymers established that the polymerization was well controlled (Figure S2 in the Supporting Information). The molecular weight distributions were narrow and therefore, relatively low polydispersity indices were obtained. Moreover, the number-average molecular weights ($M_{n,SEC}$) increased coherently with the conversion achieved in the range under study.

Because of the incorporated BODIPY groups, the terpolymers are fluorescents. As an example, Figure 1 shows the absorption and emission spectra of sample **T1** in water. According with a BODIPY-based compound, the maximum of absorption (λ_{abs}) is around 524 nm, independently on the composition and the solvent employed in its determination. This fact can be observed from the photophysical data showed in Table 1. Moreover, a good relationship could be determined between the intensity of the absorption at this wavelength and the concentration of the solution, according with the Lambert−Beer's law. Therefore, the extinction molar coefficient (ε) values in aqueous solution, collected in the table, could be estimated. These ε values are similar in the three cases and, compared with the ε value of the parent dye PM567 (77000 M^{-1} cm^{-1} in water/ethanol 90 vol. %),[33] they are low. Thus, it can be estimated that only around 5−10% of the polymer chains have covalently bounded to a molecule of dye. In addition, distinctive fluorescence, assigned to the BODIPY units, appears at 500−650 nm. The maximum emission (λ_{em}) takes place at around 535 nm. Moreover, the quantum yields (Φ_{flu}) are quite high, according with the typical values obtained for BODIPY-based polymers.[41,42] We can conclude that the photophysical properties of the new P(MEO_2MA-*co*-OEG_{8-9}MA-*co*-BODIPYMA) terpolymers are not affected by the differences in the terpolymer composition at low temperature (around 10 °C), observing similar values for λ_{abs}, ε and λ_{em} in water (PBS pH = 7, μ = 0.1 M).

The methacrylic polymers with oligo(ethylene glycol) side chains under study are thermo-sensitive.[22−25,34−40] They undergo a thermal transition in water solution, becoming reversibly nonsoluble when the solution temperature is higher than the LCST. The LCST values (cloud points) of each polymer were determined by turbidimetry and they are collected in Table 1. As it is well-known, these values linearly increase when the MEO_2MA ratio in the polymer decreases.[23−27] But the most important issue here is that the system shows a remarkable increase in the fluorescent intensity when the polymer collapses, which means at temperatures above the LCST. Thus, Figure 2 shows the dependence of the absorbance at 600 nm (cloud point determinations) and the quantum yield of fluorescence on the temperature for each polymer water solution. At low temperature, the polymers are soluble (transparent) and only relative weak fluorescence is observed. However, the fluorescence intensity increases drastically when the thermal transition is achieved. According with Wang et al.,[20] this fluorescence enhancement is due to the aggregation properties of the thermo-sensitive polymer and moreover, a covalent bond between the BODIPY units and the polymeric chains is necessary for the heat-induced fluorescence enhancement. The difference in the quantum yield above the cloud point between the sample **T1** (Φ_{flu} close to the unit) and the rest of samples could be explained from the differences between the macromolecular mobility. Thus, polymers with longer ethylene glycol side chains (samples **T2** and **T3**) exhibit higher mobility above this transition.

Apart from the relevant change in the optical and luminescent properties of the polymers in water solution at the LCST, it is also important to point out that this change is reversible. In order to prove this fact, Figure 3 shows the change in absorption at 600 nm and quantum yield emission of sample **T1** for different heating/cooling cycles. The changes in absorbance (transparent/opaque) and in fluorescence (enhancement/quenching) occurred reversibly at least six times. In summary, a relevant increase in the fluorescence intensity can be achieved at the desired temperature in a reversible way. This fact is due to the LCST of these terpolymers can be tuned just varying the feed monomer composition in the synthesis.

P(MEO_2MA-*b*-BODIPYMA) Block Copolymers. In the previous terpolymeric system, the ratio of [MEO_2MA+$OEG_{8-9}MA$]$_0$ /[BODIPYMA]$_0$ was 1400:1 in all cases. This

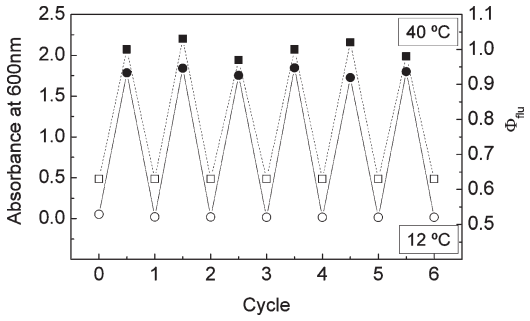


Figure 3. Change in absorbance at 600 nm (circles) and in fluorescence quantum yield (squares) of sample **T1** dissolved (0.36 mg mL^{-1}) in PBS (pH 7, μ = 0.1 M), where the temperature was changed repeatedly between 12 °C (< LCST, open symbols) and 40 °C (> LCST, full symbols).

suggests that inter- or intramolecular association of the BODIPY units scarcely occurs and therefore, BODIPY units should be isolated from each other. However, it was demonstrated that $\pi-\pi$ stacked BODIPY units can play an important role in the emission properties.[15] In order to study this effect in the thermo-sensitive system under study, P(MEO_2MA-*b*-BODIPYMA) diblock copolymers were synthesized in two steps, according to the procedure described in the experimental part and shown in Scheme 2. In the first step, a thermo-sensitive segment of P(MEO_2MA) was prepared in a controlled way ($M_{n,SEC}$ = 19686 g mol^{-1}, M_w/M_n = 1.41) and used as macroinitiator for the ATRP of BODIPYMA in a second step. The synthetic conditions and the properties of the obtained diblock copolymers are summarized in Table 2. It should be noted that copolymers were mainly and deliberately constituted by MEO_2MA. Therefore, their cloud point values almost do not change because the BODIPY blocks are very short in comparison. In fact, monomer conversions could not be determined gravimetrically. In addition, the ^1H NMR signals corresponding to the chromophore units are not visible in their spectra due to its low relation proportion. However, the incorporation of BODIPY units could be observed from the color of the polymers but also, by SEC determinations. An increment in the molecular weights was observed without apparent detriment of the narrow polydispersity indices. Thus, the BODIPYMA content in samples **B1** and **B2** was estimated, using SEC measurements, as 1.7 and 2.8%, respectively. These values were confirmed from the experimentally calculated molar extinction coefficients.

The photophysical properties of the diblock copolymers were evaluated and some relevant differences in comparison with the terpolymers were observed (Table 2), taking into account that the dye content in the block copolymers is higher than in the terpolymers. The typical absorption spectrum of BODIPY dyes has a main absorption band, attributed to the 0−0 vibrational band of a strong S_0-S_1 transition (~520 nm), and a shoulder assigned to the 0−1 vibrational band of the same transition (~490 nm). As can be seen in Figure 1, the relative intensity of these absorption bands in PBS is different in the block copolymers where the 0−1 vibrational band increases. In addition, that change in the absorption band is accompanied by a strong decrease in the fluorescence quantum yield until Φ_{flu} = 0.1. This fact was first observed by Johansson et al.[43] and was interpreted as a rare example of aggregation in BODIPY dyes. The H aggregate with a main band around 477 nm was confirmed later by Nagai et al.[21a,15] in nonthermo-sensitive diblock copolymers based on a BODIPY monomer and in random

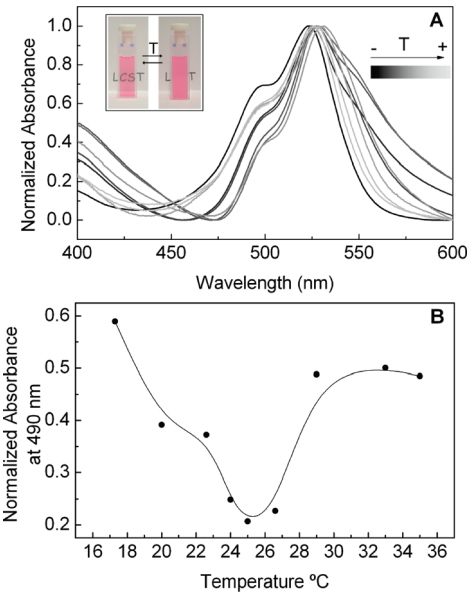


Figure 4. (A) Normalized UV−vis absorption spectra of the P(MEO_2MA-*b*-BODIPYMA) diblock copolymer (sample **B1**) dissolved (0.36 mg mL^{-1}) in PBS (pH 7, μ = 0.1 M) at different temperatures and (B) evolution of the absorbance intensity at 490 nm with the temperature. Photographs taken at temperature below the LCST (left part) and above the LCST (right part) of sample **B1** are included.

copolymers based on DMAEMA and BODIPY. There are two kinds of aggregates in chromophores depending of the molecular orientation, J-aggregates and H-aggregates. The J-aggregate is a one-dimensional molecular arrangement, in which the transition moments of individual monomers are aligned parallel to the line joining their centers (end-to-end arrangement). The most characteristic feature of J-aggregate is that it exhibits a narrow peak (J-band) red-shifted in the absorption spectrum with respect to the monomer absorption. The H-aggregate is also a one-dimensional array of molecules in which the transition moments of individual monomers are aligned parallel to each other but perpendicular to the line joining their centers (face-to-face arrangement). The absorption spectrum of the H-aggregate consists of a blue-shifted band (generally is not as narrow as the J-band) with respect to the monomer absorption. In both cases, the fluorescence decreases with the formation of aggregates.

Figure 4 shows how the intensity of the peak at 490 nm in normalized spectra (H-aggregates) changes with the temperature. It decreases with temperature until to a value close to the LCST, increasing again with a further increase of temperature. The first decrease could be explained assuming the formation of a micellar solution, at low temperature, that is progressively destroyed with the increase of temperature until the LCST. The last increment could be attributed to the thermal collapse of the P(MEO_2MA) chains in the same way as it was explained in the previous section for the random terpolymers. The hydrophobic character of the BODIPY block makes possible the formation of micelles at temperatures below the LCST. However, the whole polymer becomes non soluble above the LCST, the $\pi-\pi$ stacking between the chromophores disappears and the absorption spectrum changes.

The variation of the fluorescence spectrum and the evolution of Φ_{flu} with the temperature are plotted in Figure 5. It can be observed that the fluorescence displays the same behavior with temperature as the absorption at 490 nm. This is logical since the excitation wavelength used was exactly 490 nm. The low emission together with the characteristic

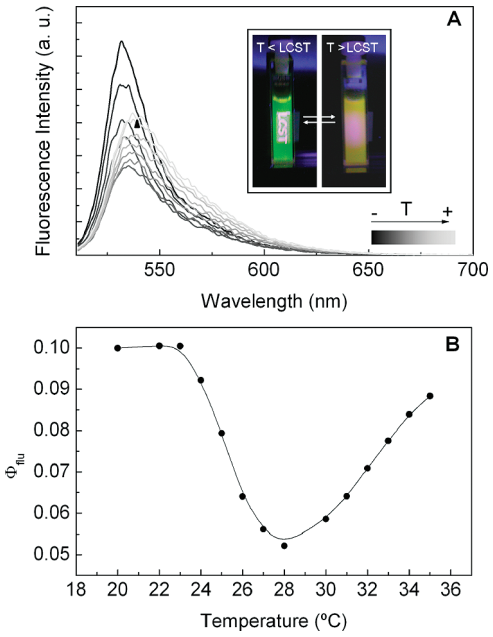


Figure 5. (A) Emission spectra (exciting at 490 nm) of the P(MEO_2MA-*b*-BODIPYMA) diblock copolymer (sample **B1**) dissolved (0.36 mg mL^{-1}) in PBS (pH 7, μ = 0.1 M) at different temperatures and (B) evolution of its emission quantum yield with the temperature. Photographs under UV irradiation taken at temperature below LCST (left part) and above LCST (right part) of sample **B1** are included.

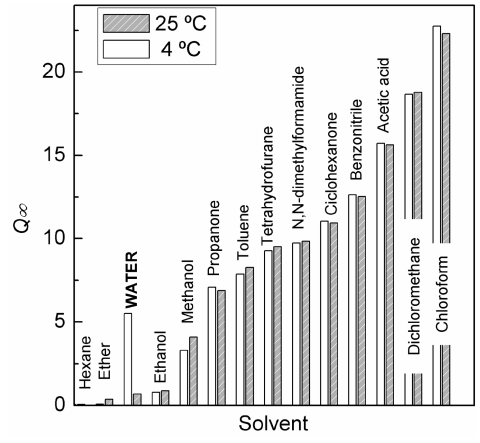


Figure 6. Equilibrium swelling of the P(MEO_2MA-*co*-BODIPYMA) hydrogel in several solvents at 4 and 25 °C.

short BODIPY's Stokes shift made impossible the measurement of the fluorescence exciting at the main absorption band.

P(MEO_2MA-*co*-BODIPYMA) Hydrogel. In order to show that the MEO_2MA-BODIPYMA system can also be interesting for other polymeric structures, a hydrogel of MEO_2MA was synthesized under the same experimental conditions previously described by our research group,[31] but adding a very low proportion of BODIPYMA (1400:1). Figure 6 shows that the equilibrium swelling of this compound depends on the solvent. It can be observed that it is not function of the temperature, with the exception of the solvent water, where a relevant variation with this parameter takes place.

This dependence is shown in detail in Figure 7, in which the equilibrium swelling (Q_∞), the emission (excited at 490 nm) and the absorbance at 600 nm spectra are plotted versus temperature. In this kind of hydrogels, a radical change in the volume and an increase in the opacity are observed above

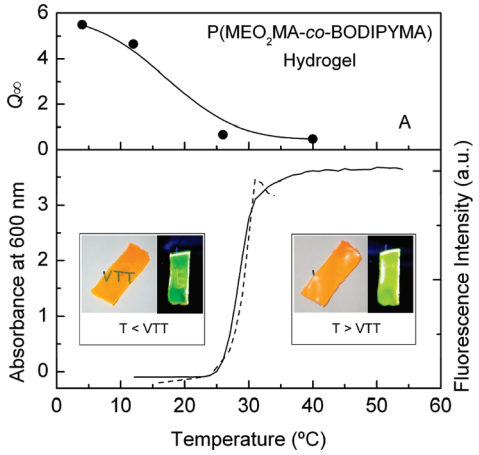


Figure 7. (A) Equilibrium swelling and (B) evolution of absorbance at 600 nm (solid line) and emission (excited at 490 nm, dashed line) of the P(MEO_2MA-*co*-BODIPYMA) hydrogel in PBS (pH 7, μ = 0.1 M) vs temperature. Photographs under visible and UV irradiation taken at temperature below VTT (left part) and above VTT (right part) are included.

a temperature value, called volume transition temperature (VTT), in the same way that the LCST in the noncross-linker polymers.[31] In this case, this transition takes place independently on the BODIPYMA units. However, the novelty of the present system is again that the hydrogel is fluorescent due to the presence of BODIPY side groups. Analogous to their homologues linear polymers, the intensity of the fluorescence depends on the temperature, as it can also be observed in Figure 7. Thus, for temperatures higher than the VTT (around 26 °C) the equilibrium swelling decreases, the system becomes opaque and the fluorescence emission intensity increases. Therefore, it is experimentally demonstrated that the cross-linked structures show the same interesting results of fluorescence observed for the linear polymers. It is important to mention that the VTT of this kind of hydrogels can also be easily modulated by the incorporation of low proportion of other oligo(ethylene glycol) side chain macromonomers, as it was found previously by our research group.[31]

Conclusions

Three different families of thermo-sensitive polymers based on MEO_2MA with a very low proportion of a fluorescent monomer based on BODIPY have been synthesized to study their thermosensitive/fluorescent behavior in aqueous solution: (i) Well-controlled random P(MEO_2MA-*co*-OEG_{8-9}MA-*co*-BODIPYMA) terpolymers, in which the monomeric feed ratio establishes the values of the LCST. In this system, the intensity of the fluorescence strongly increases in a reversible way at the LCST. (ii) Well-controlled diblock P(MEO_2MA-*b*-BODIPYMA) copolymers, in which the H-aggregation between the BODIPY units reduces the emission properties. (iii) A random P(MEO_2MA-*co*-BODIPYMA) hydrogel that becomes opaque and exhibits an increase of the fluorescence intensity rising the VTT, in the same way that its linear homologues.

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Supporting Information Available: Figures S1 (NMR spectra) and S2 (SEC traces). This material is available free of charge via the Internet at http://pubs.acs.org.

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