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Photosensitization of Bioinspired Thymine-Containing Polymers

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Here, we report a sensitization study on a family of water-soluble photopolymers based on thymine. The goal of this study was to determine whether the presence of sensitizer molecules would promote photocrosslinking/immobilization of the polymers using low-energy irradiation (520 nm) as compared to the UV irradiation (~280 nm) necessary for the standard photoinduced process to take place. With the aid of Eosin Y Spirit Soluble (EY) as a sensitizer, water-soluble polystyrene copolymers of vinylbenzylthymine−vinylbenzyltriethylammonium chloride (VBT-VBA) were immobilized after exposure to visible irradiation. By exciting the sensitizer molecule in the presence of VBT copolymers at a wavelength where absorption by the latter does not occur, the triplet state of the sensitizer is generated in high yields, and consequently, polymer photocross-linking takes place. UV−vis spectroscopy has been used to study the effect of irradiation dose, copolymer composition, and sensitizer concentration on the photoreactivity of VBT polymers. These studies demonstrate the feasibility of using Eosin Y as a sensitizer to achieve the thymine photodimer formation, resulting in immobilization of VBT-VBA-EY films on PET substrate. This provides complementary information on photoinduced immobilization of VBT-VBA films that are crucial for developing new classes of environmentally benign materials and new energy-saving methods.

1. Introduction

More often than ever, governments, industries, and researchers across the world recognize that environmentally benign alternatives for the currently used toxic materials and new energy-saving processes have to be explored.[1] A smart way to do it will be mimicking what “mother nature” does.[2] High molecular weight biological molecules (proteins and nucleic acids) contain functional groups, which are able to undergo photoreactions.[3] Nucleic acid residues, so-called bases, have a high absorbance in the UV region. Moreover, because of the rigid DNA or RNA structure, the bases are aligned parallel to each other, allowing the overlap of the electronic orbitals. Back in 1941, it was first reported that DNA could be damaged by exposure to UV light.[4,5] As a result of this photoreaction, the double carbon−carbon bond in thymine moieties cyclizes with an adjacent thymine, forming a cyclobutane thymine dimer. It was later discovered that the dimerization of adjacent thymine bases in the DNA strands is a major factor in biological inactivation of exposed DNA.[6] Genome damage is considered to be the most important event for many changes of biological significance (photocarcinogenesis and photogenotoxicity).[7]

During the last decades, extensive research has been done to design synthetic polymers containing nucleic acid bases.[8,9] In light of bioinspiration, we have designed a synthetic monomer (4-vinylbenzyl)thymine (VBT)[10–25] that is especially interesting due to its ability to photocross-link upon irradiation with the short-wavelength ultraviolet component of sunlight.[26,27] The chemistry of the cross-linking and immobilization of these bioinspired photoresists has been derived from a simple photochemical transformation that occurs in nature. The specific process is the $2\pi + 2\pi$ photodimerization of thymine base units within DNA.[26,27]

The homopolymer of VBT is insoluble in water due to its strong intermolecular interactions (mostly hydrogen bonding). However, copolymerization of VBT with either cationic- or anionic-substituted styrenes, such as vinylbenzyltriethylammonium chloride (VBA) or vinylphenyl sulfonate (VPS), results in water-soluble copolymers with the desired solubility. In a typical experiment, the VBT-containing copolymer is deposited from aqueous solution onto compatible substrates, the solvent is allowed to evaporate, and the resulting dry polymer film is cross-linked by UV light irradiation. Following cross-linking, the copolymer undergoes a transition from individual polymer chains to an insoluble cross-linked network, where the polymer chains are connected to each other through thymine cyclobutane photodimers, giving rise to a macromolecule with “infinite” molecular weight. Transitions from individual chains to one cross-linked system are generally associated with a variety of changes in the polymer physical properties, such as elasticity, tensile strength, and solubility.[28]

The adaptability of VBT makes it a very attractive monomer, because the balance between photoreactivity, solubility, and noncovalent interactions can be fine-tuned for a wide variety of applications. In addition, the existence of natural mechanisms to reverse the photodimerization reaction and to restore the polymers to their original state provides exciting opportunities to create fully recyclable materials.[20] Some practical applications involve hair-styling products,[17] electrically conductive coatings,[19] antibacterial-coated surfaces,[18] controlled release drug

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delivery systems,[23,29] recyclable plastics,[16,20] and photoresists[11–14,24] including fabrication of electronic components and printed circuit boards.[19]

The laws of thermodynamics and kinetics govern chemical processes. Every transformation requires an input of energy to overcome the activation energy barrier of the transition state. These energy inputs can scale up to be a substantial component of the overall environmental impacts of a transformation. New methods/transformations need to be designed to work within more readily accessible energy limits. To minimize the amount of energy necessary to cross-link the VBT polymers (currently UV irradiation) and to make the curing process safer and cheaper, sensitizer molecules can be added to the copolymer to photodimerize the VBT with visible light. Preliminary studies showed that adding a strongly UV-absorbing model guest species in different quantities, even exceeding the amount of thymine in the polymer, did not hinder photocross-linking. Films containing water-soluble thymine-based photopolymer and various dyes were successfully immobilized on a substrate by exposure to ultraviolet irradiation.[25]

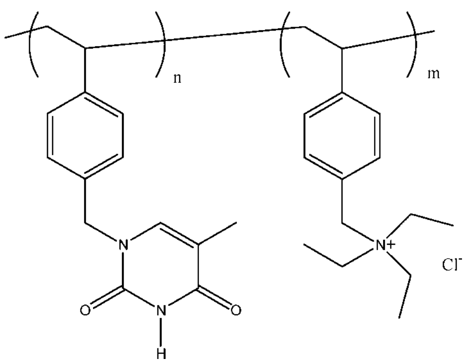
Previous studies on sensitization of thymine in DNA showed that some sensitizers are able to photoinduce the cyclodimerization of thymine through a triplet energy transfer mechanism.[30–33] Furthermore, it was shown that thymine is the nucleotide with the lowest triplet energy,[34] being the most favorable acceptor position for an energy transfer mechanism. On the other hand, Eosin Y Spirit Soluble (EY) is an attractive sensitizer because it is extremely sensitive and efficient in the visible region of the electromagnetic spectrum ($\lambda_{max} = 535$ nm and $\epsilon_{540} = 2600$ L mol^{-1} cm^{-1}).

In this context, we report here some interesting results obtained in sensitization experiments on three photocross-linkable polymers with different copolymer ratios, (VBT)(VBA), (VBT)(VBA)$_4$, and (VBT)(VBA)$_8$, at room temperature using EY as a sensitizer. A noteworthy feature of this sensitized photodimerization process is the fact that it can be carried out at wavelengths (520 nm) where the reverse reaction (ring opening) does not occur, meaning that a dimer is formed from every two neighbor thymines. We are interested in determining whether the EY triplet present in the system would be able to sensitize a thymine moiety in the copolymer. In the course of this work, fundamental issues such as the irradiation time dependence, the sensitizer concentration dependence, and the copolymer ratio effect were investigated. Additionally, with the increasing attention given to the environmental and toxicological implications associated with commercial materials and the high-energy inputs required for most existing processes, sensitization of thymine based polymeric systems is consistent with the principles of green chemistry:[1] a nontoxic, water-soluble, and biodegradable material, derived from renewable feedstock and that requires low energies for its processing.

2. Experimental Section

2.1. Materials and Methods. All chemicals, unless otherwise specified, were purchased from Sigma-Aldrich in their purest available form and used as received. VBT and VBA were synthesized as described previously.[10] NMR spectroscopy was performed on a Bruker 250 MHz spectrometer. The hydrophilic-treated side of PET-X4C1 film base (Dupont) was used without preparation. Wire-wound milled coating rods were obtained from R. D. Specialties Inc. (Webster, NY). Visible light irradiations were performed using a 50 W halogen lamp (OSRAM, Halostar Standard with UV Filter, model 64440) followed by an interference filter $\lambda_{max} = 520$ nm (part #43-173, Edmund Optics) to eliminate any ultraviolet-induced photocross-linking. UV−vis measurements were performed on a Perkin Elmer UV−Vis Spectrometer Lambda 20.

SCHEME 1: (VBT)$_n$(VBA)$_m$ Copolymer



2.2. Synthesis of (VBT)$_n$(VBA)$_m$ Copolymer, where $n = 1$ and $m = 1$, 4, or 8. VBT was copolymerized with VBA in various molar ratios (Scheme 1). The copolymerization reactions were carried out as follows. Isopropyl alcohol (IPA) was used as the solvent, heated in hot bath to 85 °C while stirring at 200 rpm under a N_2 atmosphere. To the IPA was added VBA (MW = 253.82 g/mol) followed by VBT (MW = 242.28 g/mol). After the solution became clear yellow, the temperature was decreased to 65 °C and 2,2-azobisisobutyronitrile (AIBN) (1% w/w) was added. After 16 h, the clear yellow solution was cooled to room temperature and concentrated to ~60% volume by rotary evaporation. The viscous yellow solution was added to stirring acetone, precipitating the copolymer as a white solid. After 2 h of stirring, the white precipitate was filtered and dried under vacuum, producing a fine white powder.

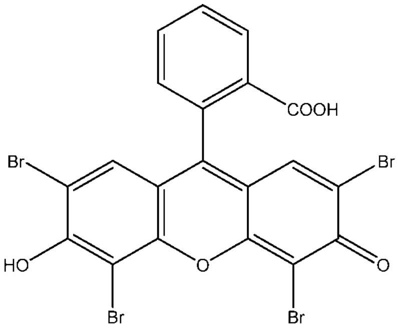
The vinyl group of unreacted monomers has a distinctive ^{1}H NMR spectrum due to the two spin systems corresponding to the terminal protons of the vinylic double bond. Taking a solution (D_2O) NMR spectrum would show a doublet of doublets separated by approximately 160 Hz, at chemical shifts between 5 and 6 ppm. In our case, ^{1}H NMR spectroscopy confirmed the absence of unreacted monomers in the synthesized polymers.

2.3. Preparation of VBT-VBA-EY Solutions. Solutions of each (VBT)(VBA)$_m$ copolymer were prepared as follows: 1 g of polymer [either (VBT)(VBA), (VBT)(VBA)$_4$, or (VBT)(VBA)$_8$] was dissolved in 10 mL of deionized water. Subsequently, aliquots of ethanol solutions of EY (between 10 and 60 μL) were added to the VBT-VBA aqueous solutions, to get the corresponding concentrations, namely, (VBT)(VBA)$_m$-EY$_k$ where $m = 1$, 4, or 8 and $k = 0.2$, 0.5, or 0.9. Mixed solvents containing ethanol increased the solubility of EY. The resulting mixtures were stirred until homogeneous solutions were obtained. The molecular structure of the EY (MW = 647.92 g/mol) is displayed in Scheme 2.

2.4. VBT-VBA-EY Coatings and Irradiations. A thick layer of solution of VBT-VBA-EY was coated onto the hydrophilic-treated side of PET film using a #03 wire-wound coating rod (wet film thickness ≈ 6.8 μm).[35] The films were allowed to dry under ambient conditions for 1 h and then at 80 °C for 1 h. After drying, the thickness of VBT-VBA-EY film was expected to be about 680 nm.[24] Special care was taken to minimize exposure of the films to visible light by working in the dark.

The VBT-VBA-EY films coated on the PET substrate were irradiated with visible light of $\lambda = 520$ nm through a patterned opaque mask from a distance of 15 cm. The films were exposed

SCHEME 2: EY



to various doses of visible light ranging from 0 to 180 min. After irradiation, the films were rinsed by immersion into deionized water for 60 s to remove the nonimmobilized polymer and sensitizer. The photoimmobilized coatings were dried at room temperature, and in many cases, the resulting patterns were visually observed due to the EY presence. The UV−vis spectra of the dried exposed films were measured.

3. Results and Discussion

It is known that VBT-VBA polymers in areas exposed to UV light (~280 nm) undergo photocross-linking due to dimerization of thymine pendant groups.[10–14] The UV−vis absorption spectrum of aqueous solution of (VBT)(VBA)$_4$ polymer (5 mg/L) is shown in Figure 1a. It can clearly be seen that the colorless VBT-VBA polymer does not absorb light neither at $\lambda \sim 535$ nm, where the EY sensitizer has its maximum absorption (Figure 1b), nor at $\lambda \sim 520$ nm, where the visible irradiation is taking place. Moreover, the PET film used as a substrate is transparent and absorbs only in the deep ultraviolet region of the spectrum ($\lambda < 300$ nm), not affecting the visible absorption profile. Therefore, the EY sensitizer absorbed virtually all of the exciting light.

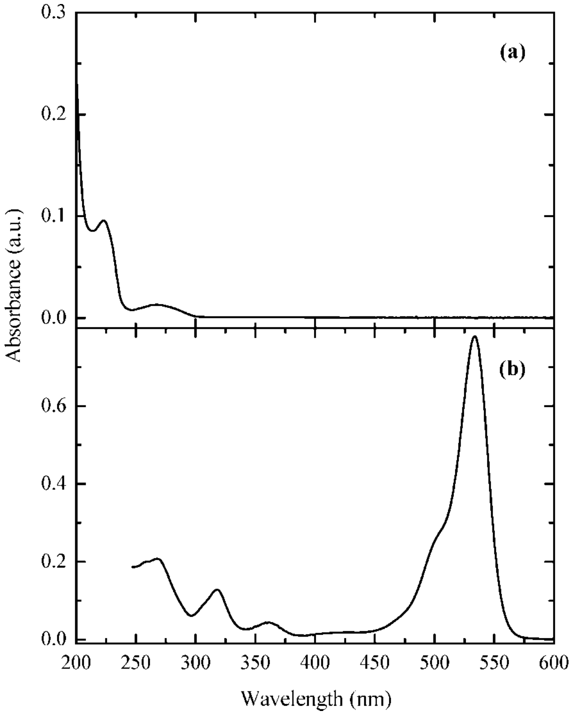


Figure 1. Absorption spectra of (a) (VBT)(VBA)$_4$ polymer in water 5mg/L and (b) EY in ethanol.

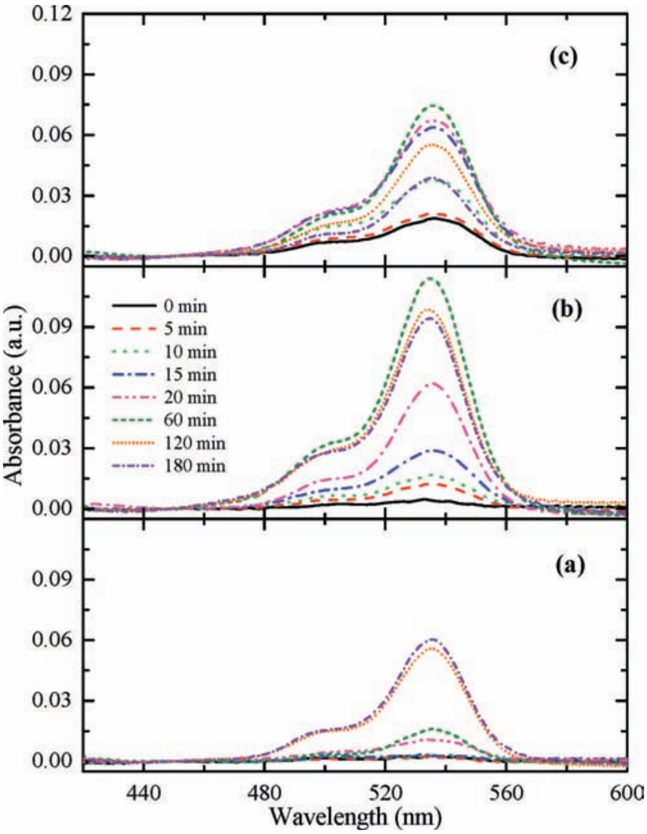


Figure 2. Absorption spectra of the films of (a) (VBT)(VBA)$_1$-EY$_{0.2}$, (b) (VBT)(VBA)$_1$-EY$_{0.5}$, and (c) (VBT)(VBA)$_1$-EY$_{0.9}$ after exposure to various irradiation times from 0 to 180 min and immersion into deionized water for 60 s.

3.1. Copolymers (VBT)(VBA)$_1$-EY$_k$ where $k = 0.2$, 0.5, or 0.9. The UV−visible spectra of (VBT)(VBA)$_1$-EY$_k$ ($k = 0.2$, 0.5, or 0.9) films coated on a PET substrate, exposed to various doses of visible light irradiation (520 nm) and rinsed with water, are shown in Figure 2a−c. It can be seen that the development and reduction of the peak at 535 nm corresponding to the EY absorption can be observed; therefore, it is reasonable to assume that the EY-mediated photocross-linking is taking place. The cross-linking of VBT-VBA-EY films causes the immobilization of the polymer-sensitizer on the substrate due to the high molecular weight and complicated structure of the cross-linked network.

The EY sensitizer molecules trapped in the immobilized network (by its electrostatic interactions with immobilized polymer) do not easily diffuse through the polymer matrix and are responsible for the absorption peak at 535 nm. Thus, the peak indicates the presence of a cross-linked network. The areas where the polymer-sensitizer system has not been exposed to visible irradiation can be easily washed away, in the same way as conventional negative photoresists.

As a control, samples of each copolymer containing no EY were irradiated and rinsed in an identical manner. It is interesting to note that exposure of (VBT)(VBA)$_m$ ($m = 1$, 4, or 8) copolymers to 520 nm light, in the absence of the photosensitizing EY, did not result in any detectable cross-linking/insolubilization of the polymer, even after 180 min of irradiation [Figure 3 (●)]. It can be rationalized by pointing out that uncross-linked VBT-VBA is water-soluble and since in the absence of sensitizer no cross-linking was expected to occur at 520 nm, only trace amounts (if any) of VBT-VBA will be immobilized on the PET substrate. To detect the immobilized

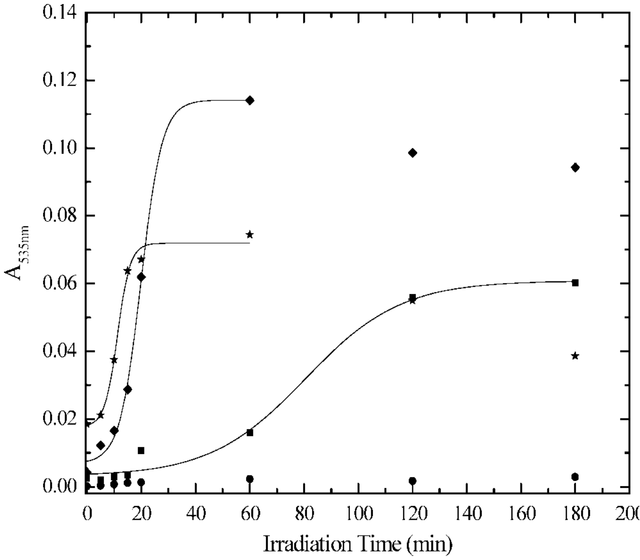


Figure 3. Time dependence of the intensity of the absorption peak at 535 nm for (VBT)(VBA)$_1$ (●), (VBT)(VBA)$_1$-EY$_{0.2}$ (■), (VBT)(VBA)$_1$-EY$_{0.5}$ (♦), and (VBT)(VBA)$_1$-EY$_{0.9}$ (★) after exposure to various irradiation times and immersion into deionized water for 60 s. The lines are intended as a guide for the eye only.

VBT-VBA (which is colorless), the samples were toned with red anionic dye (FD&C Red #3), which is known to adsorb efficiently on oppositely charged VBT-VBA.[15]

For (VBT)(VBA)$_1$-EY$_{0.2}$ irradiated for 5, 10, and 15 min, almost the entire polymer-sensitizer was removed from the surface by the aqueous wash and no increase in absorbance at 535 nm was observed. As irradiation time increases (and therefore energy dose intensifies), the cross-linking progresses further, and the polymer-sensitizer gets immobilized on the PET substrate. Figure 3 (■) shows the intensity of the absorption peak at 535 nm as function of the irradiation time, where it can clearly be seen that the peak increases after the polymer is irradiated for 20 min. However, the cross-link is not so efficient at low EY concentration even after irradiating for 180 min. This can be attributed to the fact that in the (VBT)(VBA)$_1$-EY$_{0.2}$ sample the EY concentration in the close environment of the thymine moieties is low and, therefore, is not enough to promote the photodimerization. The higher amount of cross-linking observed in samples with EY$_{0.5}$ and EY$_{0.9}$ supports this hypothesis.

In the case of (VBT)(VBA)$_1$-EY$_{0.5}$, detectable cross-linking was observed after the polymer-sensitizer system was irradiated for 5 min. At higher irradiation doses, the amount of immobilized polymer first increases and then reaches saturation and declines. Figure 3 (♦) shows that the 535 nm absorption peak reaches its maximum at 60 min of irradiation and decreases for 120 and 180 min. Such behavior was also observed for (VBT)(VBA)$_1$-EY$_{0.9}$; as the irradiation dose increases, the amount of immobilized polymer first increases, then reaches saturation between 20 and 60 min, and eventually declines for 120 and 180 min [Figure 3 (★)]. An explanation of the saturation at long irradiation times, when the photocross-linking process had finished, can be the fact that since all thymine moieties have been dimerized there are less quenchers available for the EY excited state; therefore, other photochemical pathways, such as photobleaching, might become competitive.

Immobilization of the VBT-VBA on the PET substrate was observed in all cases, but the efficiency of the process depends on EY concentration. At low EY concentration (VBT)(VBA)$_1$-EY$_{0.2}$, an irradiation time of 20 min is necessary to immobilize a measurable amount of polymer on the surface and to observe a small absorption peak. When the EY concentration increases to (VBT)(VBA)$_1$-EY$_{0.5}$ and further to (VBT)(VBA)$_1$-EY$_{0.9}$, the irradiation time necessary to immobilize the polymer on the substrate decreases to less than 5 min. The fastest rate of cure, as measured by the time to reach the maximum absorption peak in the curve, is obtained when the EY concentration is the highest (EY$_{0.9}$). On the other hand, the highest amount of immobilized polymer was observed for an intermediate sensitizer concentration (EY$_{0.5}$).

These findings are consistent with the fact that when the sensitizer concentration increases, for example, from EY$_{0.2}$ to EY$_{0.5}$, a larger amount of sensitizer excited states is produced for the same incident photon flux. Therefore, the probability of thymine photodimerization and subsequent photocross-linking increases until a saturation limit is reached, resulting in immobilization of the polymer due to higher molecular weight and complexity of the structure. On the other hand, since the lifetime of the triplet state of the sensitizer depends on the dye concentration due to self-quenching, at higher concentrations (EY$_{0.9}$), the triplet self-quenching reaction becomes an important deactivation pathway. This situation leads to fewer amounts of excited triplets available, which in turn will result in the photodimerization process becoming less efficient (see Figure 3).

3.2. Copolymers (VBT)(VBA)$_4$-EY$_k$ and (VBT)(VBA)$_8$-EY$_k$ where $k = 0.2, 0.5$, or 0.9. The same trend was observed for the other two photocross-linkable polymers with different copolymer ratios, namely, (VBT)(VBA)$_4$ and (VBT)(VBA)$_8$. Figure 4a shows (VBT)(VBA)$_4$-EY$_k$ ($k = 0.2, 0.5$, or 0.9) where an increase in irradiation time promotes the photodimerization in all cases, until saturation is reached. For this particular copolymer composition, the higher the EY concentration is, the more immobilization on the PET substrate is observed. This can be attributed to the fact that in (VBT)(VBA)$_4$ the thymine groups responsible for dimerization are further apart and more excited sensitizer molecules are needed for an observable immobilization. For (VBT)(VBA)$_8$, almost no detectable cross-linking is observable at low sensitizer concentration (Figure 4b). Only for EY$_{0.9}$, there is a small fraction of dimerization after 60 and 120 min of irradiation. This is supported by the fact that the more VBA present in the copolymer the further apart the thymine groups are; therefore, the chances for a cross-link to occur are lower. These observations also confirm that VBT is the monomer involved in dimerization/immobilization.

In all three copolymer ratios, the initial values for (VBT)(VBA)$_m$-EY$_{0.9}$ are higher than for (VBT)(VBA)$_m$-EY$_{0.5}$ and (VBT)(VBA)$_m$-EY$_{0.2}$. Affinities of the EY to the polymers and of the polymers to the substrate are responsible for nonzero absorbance at zero irradiation doses, while the difference in EY concentration (that is not soluble in water) is responsible for the difference in the initial absorbance values.

3.3. Effect of Polymer Composition on the Cross-Linked (VBT)(VBA)$_m$-EY$_k$ Films. Next, the effect of polymer composition on the sensitized thymine polymers was investigated. Figure 5 shows the sensitizer concentration dependence of the EY absorption peak at 535 nm for the three different polymer compositions. It can clearly be seen that the presence of thymine monomer is the key for the dimerization process.

The first limiting condition for the cross-linking, that is, formation of cyclobutane thymine dimer between two thymine moieties belonging to different polymer chains, is the necessity of having two such thymines in close proximity. Another point

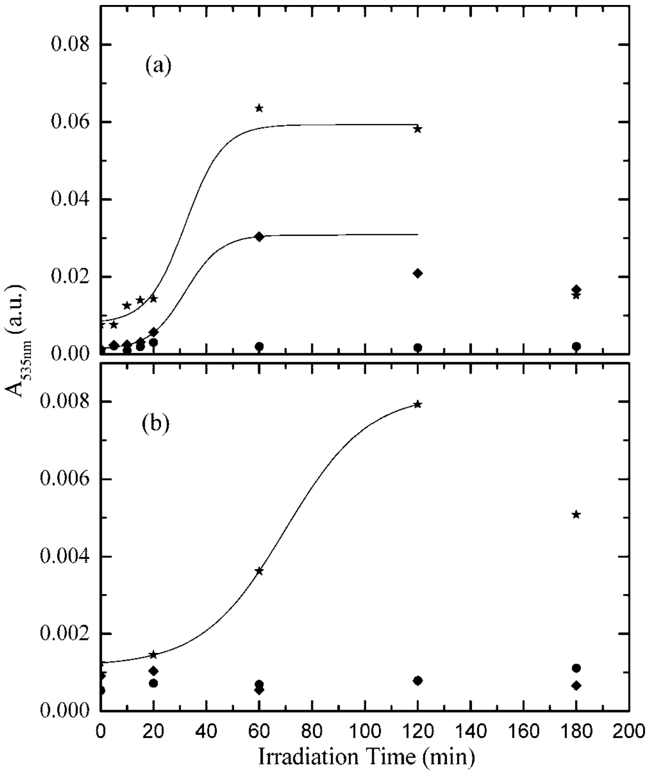


Figure 4. Time dependence of the intensity of the absorption peak at 535 nm for (a) (VBT)(VBA)$_4$-EY$_{0.2}$ (●), (VBT)(VBA)$_4$-EY$_{0.5}$ (♦), and (VBT)(VBA)$_4$-EY$_{0.9}$ (★) after exposure to various irradiation times and 60 s of development and (b) (VBT)(VBA)$_8$-EY$_{0.2}$ (●), (VBT)(VBA)$_8$-EY$_{0.5}$ (♦) and (VBT)(VBA)$_8$-EY$_{0.9}$ (★) after exposure to various irradiation times and immersion into deionized water for 60 s. The lines are intended as a guide for the eye only. Both graphs are in different vertical scales, differing by one order of magnitude.

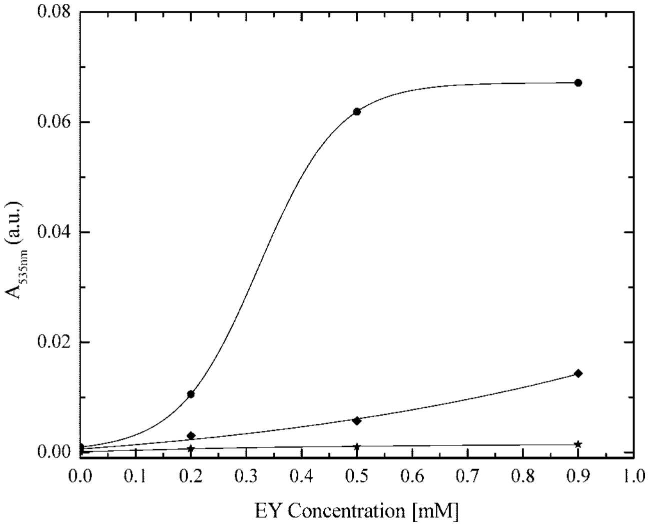


Figure 5. EY concentration dependence of the absorption peak at 535 nm for (VBT)(VBA)$_1$ (●), (VBT)(VBA)$_4$ (♦), and (VBT)(VBA)$_8$ (★) after 20 min of exposure time and immersion into deionized water for 60 s. The lines are intended as a guide for the eye only.

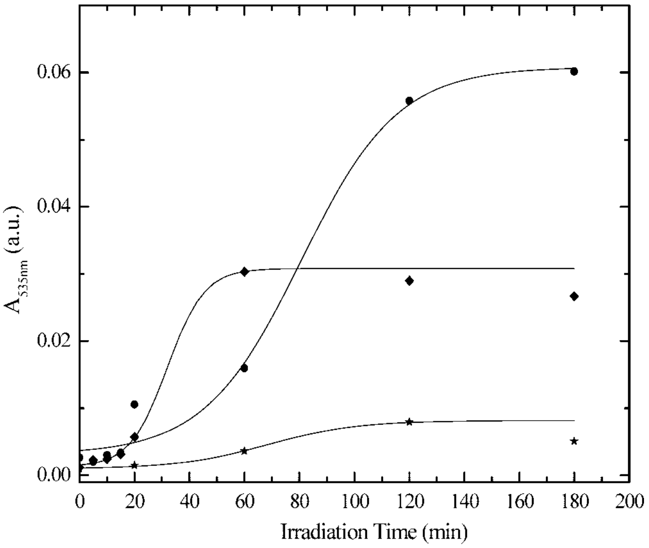


Figure 6. Time dependence of the intensity of the absorption peak at 535 nm for (VBT)(VBA)$_1$-EY$_{0.2}$ (●), (VBT)(VBA)$_4$-EY$_{0.5}$ (♦), and (VBT)(VBA)$_8$-EY$_{0.9}$ (★) after exposure to various irradiation times and immersion into deionized water for 60 s. The lines are intended as a guide for the eye only.

to be considered is the mechanism of formation of the thymine dimer. Assuming a triplet−triplet energy transfer mechanism in the generation of the dimer, since triple excitation transfer requires a collision between the donor (EY) and the acceptor (thymine), this would require a molecule of EY intercalated in the polymer chain to be located closely to a potential site of cyclobutane thymine dimer formation. The critical distance between the donor and the acceptor should be less or about 20 Å. Therefore, increasing VBA to VBT ratio will definitely decrease the chances of cross-linking. Figure 5 shows the expected trend: increasing rate of photocross-linking with increasing amount of VBT. This observation confirms the fact that the VBT monomer is directly involved in the cross-linking.

The optical densities of the EY absorption band at 535 nm after irradiation and development of (VBT)(VBA)-EY$_{0.2}$, (VBT)(VBA)$_4$-EY$_{0.5}$, and (VBT)(VBA)$_8$-EY$_{0.9}$ are presented as a function of irradiation time in Figure 6. Here, the polymer to EY ratio is kept constant (10:1), while the VBT to VBA ratio is varied between the samples. It can be seen that the sensitizer absorption increases with irradiation dose until it reaches saturation. While for (VBT)(VBA)$_4$ the saturation is reached faster than for (VBT)(VBA)$_8$ and (VBT)(VBA)$_1$, the saturation values are higher for (VBT)(VBA)$_1$.

As the irradiation time increases, the thymine groups in VBT undergo photodimerization. When the thymine groups belong to different polymer chains, the dimerization results in cross-linking. Cross-linking of the polymer chains causes a fraction of the polymer to become insoluble and stay immobilized on the surface due to increased molecular weight and acquired network structure. When all of the polymer chains are connected, the saturation is reached and continued irradiation does not cause any additional decrease in solubility. Because the fraction of the photocross-linkable VBT monomer is larger in (VBT)(VBA)$_1$ than in (VBT)(VBA)$_4$ and (VBT)(VBA)$_8$, photocross-linking of the former is more readily achieved and it reaches higher values.

3.4. Proposed Mechanism for the Sensitization of VBT Polymer by EY. Upon absorption of a photon of light, a sensitizer might undergo different competitive processes such as fluorescence, phosphorescence, intersystem crossing (ISC), energy or electron transfer, quenching to the ground state, or excimer formation. However, the most efficient one will depend on the nature of the sensitizer, the presence of other species such as quenchers, and the surrounding environment (polar or nonpolar solvents). On the other hand, not all of them proceed at the same rate due to their intrinsic nature, either unimolecular processes (fluorescence is in the nanosecond timescale while the phosphorescence is in the milisecond) or bimolecular processes.

A reasonable mechanism to explain the photodimer formation and resulting polymer insolubilization with visible irradiation involves a photosensitizer to be able to undergo a triplet−triplet energy transfer mechanism. As the feasibility of the photosensitization by triplet−triplet energy transfer process is linked to the relative excited state energies of the donor and acceptor chromophores, the triplet energies of thymine and the EY sensitizer are critical parameters in our case. The precise value of thymine triplet energy appears to be markedly different for free thymine, for polynucleotides, or for double-stranded DNA since the surrounding monomers and the presence/absence of solvent molecules will affect the conformational and structural restrictions. Obviously, the interactions between chromophores will most certainly lead to a dramatic decrease of energy levels. It is acknowledged that going from mononucleotide to polynucleotide to DNA will lower the singlet excited state energy.[36,37] Consequently, these changes will be accompanied by a lowering of the triplet state energy by an extent that is not yet known. The triplet energy of thymine was reported to be about 310 kJ/mol in free base mononucleotides on the basis of the sensitized generation of a transient dimer absorption at 380 nm by means of laser flash photolysis experiments.[34,38,39] Mononucleotides are good model systems for the corresponding base residues, but they lack the important interstrand and intrastrand interactions that are in fact present in macromolecules. In DNA, the triplet energy levels are lowered,[40] as was demonstrated that the upper limit for the triplet energy of the thymine base has been shifted down from 297 to 290 kJ/mol[33,41–43] and even lower to 270[44] and 260 kJ/mol.[45]

Our proposed sensitization mechanism involves the excitation of EY to its singlet state followed by a fast ISC. The quantum yield of EY triplet formation was reported to be $\varphi_T^{EY} = 0.8$ in water and $\varphi_T^{EY} = 0.56$ in methanol.[46] Subsequently, an energy transfer from EY in a photoexcited triplet state ($^3EY^*$) to a thymine molecule in a ground state is produced. In the next step, the resulting triplet excited thymine is expected to undergo a $[2\pi + 2\pi]$ dimerization reaction with an adjacent thymine molecule. From the reported thymine triplet energy values in DNA ($E_T^{Thy} > 260$ kJ/mol), the energy transfer from the triplet state of EY ($E_T^{EY} \approx 200$ kJ/mol)[47] to thymine in VBT-VBA polymer is expected to proceed inefficiently, since it might be “up-hill”. On the other hand, in principle, thymine dimerization can also occur via a singlet energy transfer mechanism. This possibility appears very unlikely with EY because it absorbs to longer wavelengths (530 nm) than does the VBT-VBA polymer (254 nm), so that the energy transfer from the lowest excited singlet state of EY to VBT-VBA is energetically unfavorable. Furthermore, the short lifetime of the EY excited singlet ($\sim 10^{-10}$ s) due to intersystem crossing would preclude an efficient transfer even if energetically allowed.

In contrast, in the present study of sensitizer−copolymer systems, we observed experimental evidence for a very efficient quenching of sensitizer triplets by thymine-based polymers, even when the triplet energy transfer is not energetically feasible. The difficulty in the interpretation resides in the application of previous results[33,41–45] to these dye−polymer complexes, in which the chromophores may have properties very different from those of isolated species and from DNA nucleotides. Therefore, reactions mechanisms in thymine polymeric systems cannot be completely correlated with the mechanisms in DNA strand. Further experiments are being performed to detect and document the existence of the cyclobutane dimers in sensitizer−copolymer systems. Flash photolysis and FT-EPR (Fourier transform electron paramagnetic resonance) experiments will be carried out in an attempt to understand the reaction mechanism in these biopolymers. Detection of thymine radical formation in the course of the reaction would support an electron transfer mechanism between Eosin sensitizer and thymine.

4. Conclusions

A novel class of environmentally benign photoresists based on water-soluble polymer (VBT-VBA) has been studied. The selective photoinduced immobilization of VBT-VBA-EY was studied by exposing the polymer to visible light at 520 nm. UV−vis spectroscopy has been used to investigate the effects of irradiation dose, polymer composition, and sensitizer concentration on the VBT-VBA-EY polymer films formed upon photoirradiation. Varying these parameters allows controlling the degree of cross-linking; therefore, the solubility of the polymer can be fine-tuned for a desired application. This creates an opportunity to use a system using sensitized cross-linked biopolymers for a variety of applications that need low energy irradiation.

The results presented here clearly demonstrated that EY could sensitize the photodimerization of thymine-containing polymers at room temperature. We proposed that the photosensitization process is probably achieved by energy transfer from the triplet excited state of the EY sensitizer to the ground state of the thymine in the copolymer, resulting in the formation of a triplet thymine, which undergoes photodimerization with an adjacent ground state thymine. This was also observed in previous studies on sensitization of thymine photodimerization in DNA and model systems.[30–33] Flash photolysis experiments on sensitizer−copolymer systems need to be performed in an attempt to prove this point. The study of these model systems is the first step toward a long-term goal of a full understanding of singlet or triplet precursors in energy and electron transfer mechanisms in water-soluble and environmentally benign polymers.

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