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New polyacrylates with stilbene and (S)-phenylalanine side chains for sensor applications. Synthesis and properties

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ABSTRACT

A new acrylic monomer bearing *trans*-stilbene in structure, (S)-N-acryloylphenylalanine acid 4-styryl-benzyl ester (AcPheS), was prepared to be further free radically copolymerized with (S)-N-acryloylphenylalanine (AcPhe). The resulting copolymer, poly((S)-N-acryloylphenylalanine-co-(S)-N-acryloylphenylalanine acid 4-styryl-benzyl ester) (PPheS) was characterized by ^{1}H NMR, UV–vis and FTIR spectroscopy, thermal analysis, GPC and specific optical rotation measurements. The copolymer composition was estimated from the NMR analysis, this being of 4:1 (AcPhe:AcPheS). The photobehavior of the copolymer in solution and thin film was evaluated comparatively to that of the parent monomer using UV–vis spectroscopy, and atomic force microscopy (AFM) for the polymeric film exposed to UV irradiation. Quenching of the stilbene species by purine and pyrimidine bases in DMF solution excited at 267 nm and 334 nm was evidenced, more efficiently being adenine.

1. Introduction

During the last decades, a great consideration has been paid to the design of poly(amino acid)s as a source of new degradable biomaterials [1,2] with a focus on the understanding of polymer structural factors which cause subtle distinctions in their properties and performance characteristics [3,4]. For this purpose, a number of amino acids have been used as building blocks in polymers, and consequently, the materials performed by this concept are known rather as "non-peptide amino-acid based polymers" or as "amino-acid-derived polymers with modified backbones" [5]. Among them, (co)polyacrylates, polyurethanes, polyisobutylenes, polyacetylenes or hydrogels that incorporate α-amino acids are relevant examples of polymers that exhibit markedly improved properties [6–10] including an augmented potential to form secondary structures such as α-helices and β-sheets [11,12]. Despite their structural diversity, such polymers are biocompatible and biodegradable, relatively nontoxic and easily metabolized by living tissues. All these properties make them useful for multiple applications like controlled drug delivery agents, chiral recognition stationary phases, asymmetric catalysts, metal ion absorbents, affinity based separators or optobioelectronic devices [6–10].

On the other hand, relatively less attention has been given for producing optically active copolymers through the copolymerization of an optically active unsaturated monomer with a chiral co-monomer possessing a photoactive moiety [13]. Depending on the average distance between chromophores and implicitly, of the dipole–dipole electrostatic interactions along the polymeric backbone, the achiral co-units are frequently forced through cooperative effects to adopt a molecular arrangement of the same chirality, as that assumed by the optically active co-units [13,14].

In previous papers, we reported data concerning synthesis of new bio(photo)polymers with biologically active sequences as (S)-phenylalanine [15], dipeptide [16,17], hydroxamic acids [18], ammonium/pyridinium groups [19] in tandem with the investigation of polymeric (chromo)fluorophores with triazene, stilbene or pyrene units in structure [20–26]. In the present work, we describe the synthesis of a novel optically active monomer ((S)-N-acryloylphenylalanine acid 4-styryl-benzyl ester) (AcPheS), which is further free radically copolymerized with (S)-N-acryloylphenylalanine (AcPhe) to achieve copolymers with amino acid sequences in each repeating unit and stilbene in the side chain. The photoisomerization and fluorescent emission of the stilbene unit in monomer and polymer solutions or in thin film, together with their optical properties are also investigated. The presence of photosensitive stilbene and the amino acid on the same polymeric chain creates the opportunity of synthesizing photoluminescent polymers potentially appropriate for sensor applications in biology, medicine, analytic techniques and optical devices.

2. Experimental

2.1. Synthesis of (S)-N-acryloylphenylalanine (AcPhe)

4.92 mL (60.6 mmol) of acryloyl chloride was added dropwise to a well stirred aqueous solution of (S)-phenylalanine optically pure

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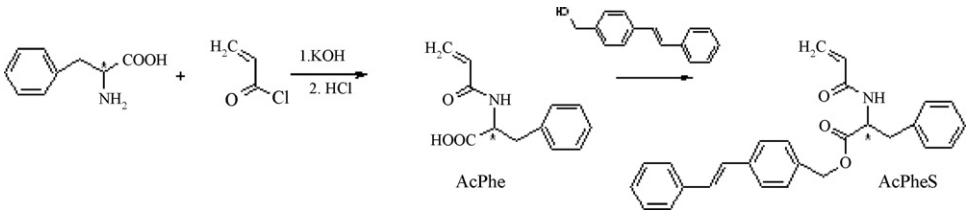

Scheme 1. Synthesis of N-acryloyl-(S)-phenylalanine (AcPhe) and (S)-N-acryloylphenylalanine-4-styrylbenzyl ester (AcPheS).

(10 g, 60.6 mmol) and potassium hydroxide (6.78 g, 121.2 mmol) in distilled water over a 30 min period, keeping the reaction mixture below 0 °C. After the complete addition the stirring was continued for other 30 min at room temperature. The mixture was acidified to pH 2 using concentrated HCl (5.02 mL, 60.6 mmol), when a dense white precipitate was separated. The resulting product was filtered and subsequently purified by recrystallization from water (melting point = 130–132 °C). Yield 3.5 g (43.85%).

Elemental analysis (%), AcPhe, calcd.: C, 65.75; H, 5.93; N, 6.39; found: C, 65.68; H, 5.90; N, 6.35.

2.2. Synthesis of (S)-N-acryloylphenylalanine acid 4-styryl-benzyl ester (AcPheS)

The esterification of (S)-N-acryloylphenylalanine (2 g, 9.1 mmol) was performed by dissolving the monomer in tetrahydrofuran (THF) and adding 1.47 g (9.1 mmol) 1.1′-carbonyldiimidazole as activation agent of the carboxylic group. The resulting mixture was stirred at room temperature for 1 h. Afterward, 1.91 g (9.1 mmol) stilbene methanol solved in THF was added and the stirring was continued at room temperature for 3 days. The crude product was precipitated in water and collected by filtration. Yield 1.8 g (61.43%).

Elemental analysis (%), AcPheS, calcd.: C, 78.83; H, 6.08; N, 3.4; found: C, 78.78; H, 6.01; N, 3.34.

2.3. Synthesis of poly((S)-N-acryloylphenylalanine-co-(S)-N-acryloylphenylalanine acid 4-styryl-benzyl ester) (PPheS)

The copolymer was obtained through the conventional free radical polymerization, the typical procedure being as it follows: 2 g (4.8 mmol) of AcPhe, 2 g (9.13 mmol) of AcPheS and 0.008 g 1,1′-azobis(cyclohexanecarbonitrile) as initiator were dissolved in 30 mL dioxane. The mixture was stirred at 60 °C and refluxed for 3 days under purified nitrogen. The resultant copolymer, poly((S)-N-acryloylphenylalanine-co-(S)-N-acryloylphenylalanine acid 4-styryl-benzyl ester), was subsequently concentrated under reduced pressure and then precipitated in diethyl ether and dried under reduced pressure at 60 °C for 48 h.

Elemental analysis (%), PPheS, calcd.: C, 75.96; H, 6.05; N, 4.06; found: C, 69.93; H, 5.98; N, 5.43.

2.4. Equipment

^{1}H NMR spectra were registered with the use of a Bruker 400 MHz spectrometer in dimethyl sulfoxide (DMSO-d_6) at room temperature with TMS as an internal standard. FTIR absorption spectra have been recorded by means of a Specord M80 spectrophotometer using KBr pellets. The UV light absorption spectra were measured at 25 °C with a SPECORD M42 spectrophotometer in DMF solution. The samples were exposed to UV irradiation using a 500-W high pressure mercury lamp without wavelength selection at room temperature. The fluorescence spectra were obtained at room temperature with PerkinElmer LS 55 spectrophotometer in DMF solution. In order to obtain the molecular weight of the copolymer the GPC chromatograms were recorded with a Polymer Laboratory MD-950 apparatus equipped with an evaporative mass detector and two PL gel 5 μm columns using DMF as solvent and polystyrene standards for calibration. The thermal transitions was performed by differential scanning calorimetry with a PerkinElmer apparatus by cooling of copolymer to 5 °C and heating at a rate of 10 °C min^{-1} up to 120 °C. Thermogravimetric analyses (TGAs) was conducted in air on a MOM (Budapest) derivatograph at a heating rate of 10 °C min^{-1}. Optical activity experiments were accomplished at 25 °C on an OPTIK Pol 1 polarimeter with cell path length of 1 dm using DMF (c = 1 g dL^{-1}). The surface morphology was investigated with a SOLVER PRO-M (NT-MDT; Russia) atomic force microscope operating in a non-contact mode. The polymeric films with a thickness of about 50 nm (appreciated with the AFM) were prepared by spin casting from DMF solution on glass plate substrate. To avoid a contamination of them, the obtained films were kept in glass petri plates under the dark up to measurements.

3. Results and discussion

3.1. Characterization

The synthesis of (S)-N-acryloylphenylalanine acid 4-styryl-benzyl ester (AcPheS) was performed *via* an acylation reaction of (S)-phenylalanine with acryloyl chloride, followed by the esterification of the resulting product (AcPhe) with 4-hydroxymethylstilbene in the presence of 1,1′-carbonyldiimidazole as carboxyl activation reagent, to produce the corresponding vinyl photomonomer (Scheme 1). The chemical structure and purity of the AcPhe and AcPheS were confirmed by elemental analysis, ^{1}H NMR and FTIR spectroscopy. The ^{1}H NMR spectrum of AcPheS monomer shown in Fig. 1, in which can be identified resonance signals attributed to the specific functions. Further observation of the spectrum of AcPhe (not shown) indicates the existence of a singlet peak centered at 12.8 ppm assigned to the carboxyl proton from the amino acid, while the NH proton can be recognized as doublet at 8.45 ppm. The

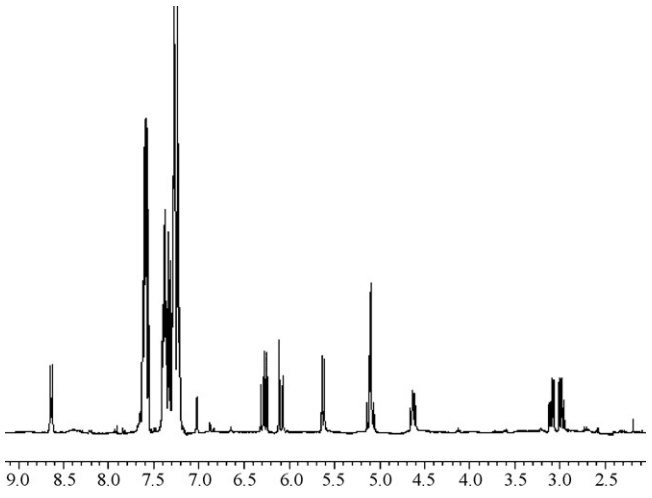

Fig. 1. ^{1}H NMR spectra of (S)-N-acryloylphenylalanine acid 4-styryl-benzyl ester.

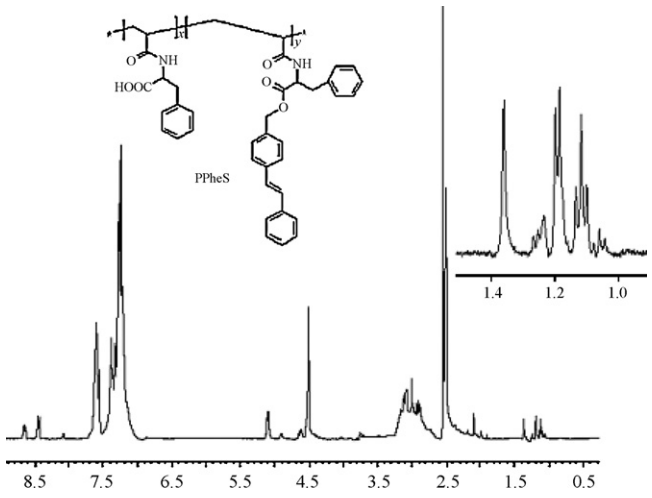

Fig. 2. ^{1}H NMR spectra of poly((S)-N-acryloylphenylalanine-co-(S)-N-acryloylphenylalanine acid 4-styryl-benzyl ester).

aromatic protons from phenylalanine provide a multiplet signal at 7.22 ppm, and the methyne proton adjacent to the carboxyl function become visible as multiplet peak at 4.5 ppm. The methylene protons from Phe give also a multiplet signal in the zone 2.9–3.36 ppm, while at 6.09 ppm is observable the doublet peak due to the C*H*–CONH olefinic proton. Other signals belonging to CH_2=CH– protons in *cis* or *trans* configuration are positioned at 5.6 ppm (doublet) and 6.28 ppm (multiplet), respectively. Each peak in the spectrum of (S)-N-acryloylphenylalanine acid 4-styryl-benzyl ester (AcPheS) confirms also the expected structure. Comparison of the AcPhe and AcPheS NMR spectra showed that in the latter appear supplementary resonance signals situated at 7.4 ppm and 7.6 ppm corresponding to the olefinic and aromatic protons from stilbene moiety. Moreover, the absence of the peak ascribed to the carboxyl proton, sustains the complete esterification of AcPhe. The doublet peak allocated to the acrylic proton (CH) appears at 6.1 ppm, while the signals assigned to CH_2=CH protons in *trans* or *cis* configuration are localized at 6.3 ppm and 5.6 ppm, respectively. Other specific signals are positioned at 8.65 ppm (NH amidic proton), 7.3 ppm (phenylalanine protons), 5.1 ppm (methylene protons from stilbene derivative), 4.5 ppm (methyne proton attached to the ester function) and 3.0 ppm, the latter being attributed to the methylene protons from phenylalanine.

The FTIR spectra of the above commented acrylic monomers illustrate absorption bands that sustain the anticipated structure as it follows: in case of (S)-N-acryloylphenylalanin*e* (not shown) there are absorption bands that correspond to the carbonyl stretching at 1651 cm^{-1} (amide I), NH band at 1535 cm^{-1} (amide II) and an additional one at 1712 cm^{-1} due to the carboxyl function arising from amino acid units. However, the presence of stilbene sequence in the AcPheS structure is confirmed through the supplementary inflexions provided by the double bond from the stilbene molecule placed at 1627 cm^{-1} and 899 cm^{-1} respectively, beside those encountered frequently in AcPhe.

Starting from the above monomers, AcPhe and AcPheS (feed ratio: 1.9:1), a classical radical polymerization in the presence of 1,1′-azobis(cyclohexanecarbonitrile) was carried out in order to obtain a copolyacrylate containing side chain stilbene moieties. The ^{1}H NMR spectra of poly((S)-N-acryloylphenylalanine-co-(S)-N-acryloylphenylalanine acid 4-styryl-benzyl ester) (PPheS) given in Fig. 2, exhibit signals characteristic for both parent monomers with exception of the typical peaks of unsaturated protons from acrylic units positioned between 5.6 ppm and 6.1 ppm. Additionally, the resonance signals localized at higher field of about 1.1–1.3 ppm correspond to the methylene protons from the acrylic copolymer. The absence of signals for unsaturated acrylic protons indicates a complete polymerization of the aforementioned monomers. From the area ratio of the peak integrals of methyne proton (4.5 ppm) attached to the carboxyl and methylene protons (5.1 ppm) from the ester function for AcPhe and AcPheS respectively, the molar ratio between the two monomers was determined, its value being 4:1.

Investigations on the thermal stability of the synthesized copolymer achieved by TGA, allows us to conclude that decomposition occurs in three distinct steps. The first stage of thermal degradation starts at 130 °C and is marked with a regular loss in mass of 1.2% up to 165 °C. In the second step of the process, in temperature range 165–225 °C, the weight loss is about 3.6%, while the major mass loss of about 54.8% takes place in the last stage of degradation (225–425 °C). The DSC thermogram evidenced a single glass transition at 115.86 °C. In order to estimate the molecular weight of the poly((S)-N-acryloylphenylalanine-co-(S)-N-acryloylphenylalanine acid 4-styrylbenzyl ester) GPC measurements were performed in DMF calibrated against polystyrene standards. The attained value for the copolymer is about 44,000, while the polydispersity index (Mw/Mn) is 1.34. This photopolymer is soluble at room temperature in common organic solvents like DMF, DMSO, 1,4-dioxane and THF.

The specific optical rotation measurements for AcPheS in DMF solution revealed a value of $[\alpha]_D^{20} = -85^\circ$ which is in agreement with the negative value $[\alpha]_D^{25} = -33^\circ$ of (S)-phenylalanine. The fact that in case of the copolymer the same value (-85°) was obtained suggested that the polymerization reaction does not affect the chirality of the asymmetric carbon present in (S)-phenylalanine moiety, for the numeric difference being responsible probably the solvent.

3.2. Trans–cis photoisomerization of the stilbene moiety

One of the most notable and well-known properties of stilbene derivatives is the *trans–cis* photoisomerization process induced by UV or visible light, which has served as a prototype for many reactions that involves 180° internal rotation around the carbon–carbon double bond [27]. Consequently, the application of stilbene derivatives in reversible optically switching processes is limited due to the unwanted side reactions. In particular, it has been shown that *cis*-stilbene can undergo a photochemical ring closure to 9,10-dihydrophenanthrene [28] or a photochemical dimerization with the formation of [2 + 2] cycloadduct *via* a singlet excimer, but unfortunately, the control of the latter structures which absorb below 280 nm is restricted. Furthermore, the isomerization to *cis* configuration causes loss of fluorescence properties. Although stilbenes are highly photosensitive species, there is not known any complete theory to elucidate the manner in which the photochemical and photophysical processes are connected to polymer properties. In order to establish the mode in which this process is influenced by the molecular architecture, it was comparatively studied the photochemical response of our stilbene monomer (AcPheS) and of resulting copolymer (PPheS) under UV-light irradiation (λ = 365 nm) in solution and thin film. Thus, upon exposure to UV light the *trans* isomer is indeed converted to *cis* conformer, the process being reflected in UV spectra by a gradually decreasing of all absorption maxima. For the AcPheS in DMF solution there was observed a diminution of the absorption maximum in the UV spectrum (not shown) with irradiation time, until the photostationary state is attained. The stilbene monomer presents four characteristic absorption bands with a maximum at 315 nm assigned to π–π^* transition, and three shoulders at 285 nm, 300 nm and 330 nm, respectively. During the spectral measurements, the appearance of two isosbestic points (346 nm and 272 nm) indicates that the

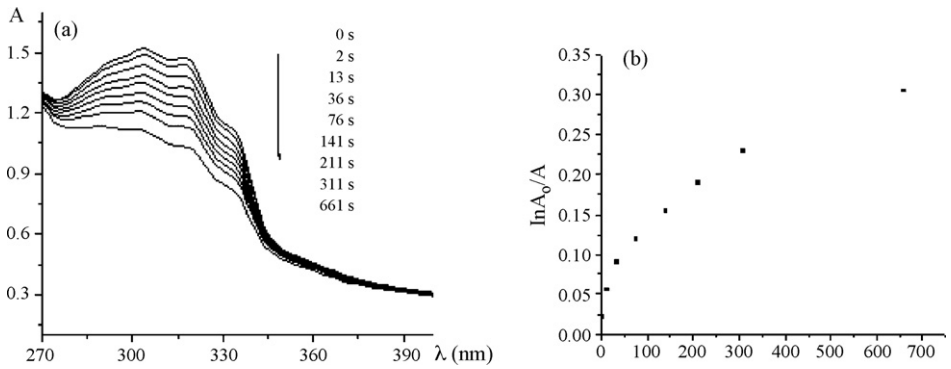

Fig. 3. The *trans–cis* photoisomerization of poly((S)-N-acryloylphenylalanine-co-(S)-N-acryloylphenylalanine acid 4-styryl-benzyl ester) in film (a) and kinetic data (b).

trans–cis photoisomerization of the stilbene molecule is the major reaction. Similar profile could also be seen in case of copolymer solution in DMF, while for the thin film cast onto quartz slide, a 3–4 nm red shift was observed. It should be noticed that the absorption maximum from 315 nm is decreasing monotonously up to a limit in favor of the *cis* isomer formation until the equilibrium between *cis* and *trans* forms is accomplished.

The photochemical isomerization is quantitatively described through a constant rate value given by the expression $\ln(A_o/A_t) = k$, where A_o and A_t corresponds to the absorbance of the *trans*-stilbene moiety before and after irradiation for a time *t*. Monitoring the changes of the absorption band of (S)-N-acryloylphenylalanine-4-styryl-benzyl ester solution the equilibrium state is reached in 30 s of irradiation ($k = 5.7 \times 10^{-2}\ s^{-1}$), when the molar fraction of *cis* isomer attains a value of 0.64 through a first order kinetics. Following the behavior of the polymer solution to UV irradiation, a gradual decrease of the absorption maximum centered at 315 nm with the irradiation time was detected (not shown), the reaction occurring after a kinetics as that found for monomer. Consequently, the rate constant of the photoprocess is $k = 4.29 \times 10^{-2}\ s^{-1}$ and the stationary state is achieved after 50 s of irradiation. The composition of stationary state in *cis* isomer is about 0.49. Under the same experimental conditions, there was pursued the behavior in thin film, for which the rate constant value calculated was $k = 1.46 \times 10^{-2}\ s^{-1}$. In this case, the equilibrium state is completed in 661 s of irradiation and the molar fraction of *cis* isomer is about 0.26 (Fig. 3). A comparison between all the kinetic plots allows us to conclude that in the film the *trans–cis* photoisomerization process is much slower than that found in the polymer solution due to the local environment of the chromophore and implicitly, its mobility significantly diminished in the film.

To facilitate the obtaining additional information on the mechanism of this process in the polymeric film based on PPheS, we have chosen to follow the *trans–cis* photoisomerization of the stilbene moiety through the atomic force microscopy (AFM) technique, the images being registered in different points of the sample to check the reproducibility of them. Fig. 4 presents AFM images of the thin films before (a) and after (b) reaching the equilibrium state. The structure of the film corresponds to the morphology of compact particles relatively homogeneously distributed on the entire substrate with no tendency for agglomeration. Additionally, it was observed that the non-irradiated surface displays a small number of hills with a height of about 8.9 nm placed in a smooth structure with average roughness less than 0.44. In fact, these hills coexist with white domains that can be related to the crystalline part of the copolymer disseminated into an amorphous matrix. After prolonged irradiation with ultraviolet light (20 min) at room temperature, a number of cone-shape structures with height of 197 nm and the base ca. 50–150 nm are optically induced in the PPheS film. In this case, the average roughness significantly increased to 11.32 nm. Obviously, the morphology of the polymeric surface changes remarkably on UV irradiation, owing to the formation of three-dimensional structures generated of the photoisomerization of stilbene copolymer in thin film. Such a result revealed that intermolecular interactions that become visible even at a lower content of fluorophore attached on the copolymer backbone (molar fraction: 0.2) but subjected to light would be responsible of the formation of hydrophobic nanodomains on the photosensitive film surface. In our opinion, it seems reasonable to assume that the new formed cone-shaped structures may be associated with J-aggregates, photoirradiation acting as main promoter for this type of self-organization within the above domains. Similar morphological changes induced after irradiation of the film were observed in case of azo-polyacrylates [29].

3.3. Fluorescence properties

All the fluorescence studies were performed by using two different excitation wavelengths, 267 nm specific for purinic and pyrimidinic bases like adenine respectively, thymine and cytosine, which will be subsequently used as quenching agents for the

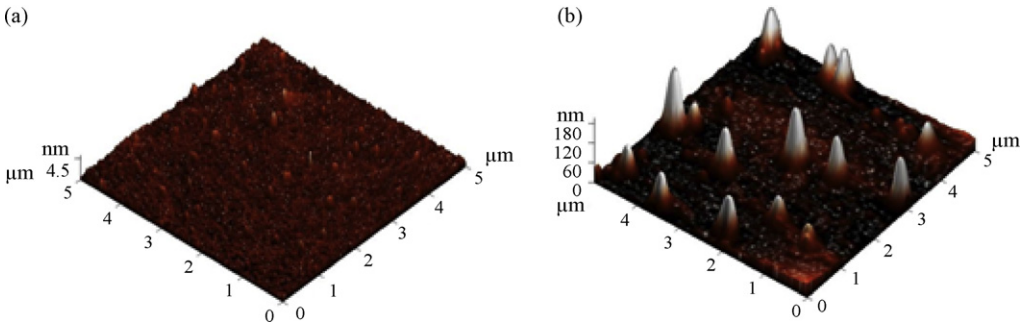

Fig. 4. 3D AFM images of the poly((S)-N-acryloylphenylalanine-co-(S)-N-acryloylphenylalanine acid 4-styrylbenzyl ester) in thin film on glass before (a) and after UV irradiation (b).

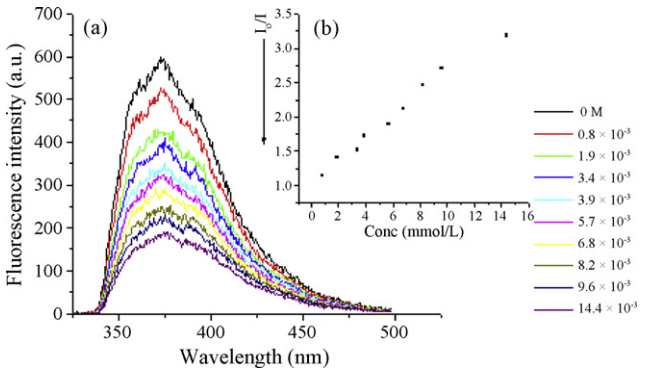

Fig. 5. Fluorescence spectra of AcPheS in DMF solution in the absence and presence of cytosine at different concentrations (a) and Stern–Volmer plot (b) λ_{ex} = 267 nm.

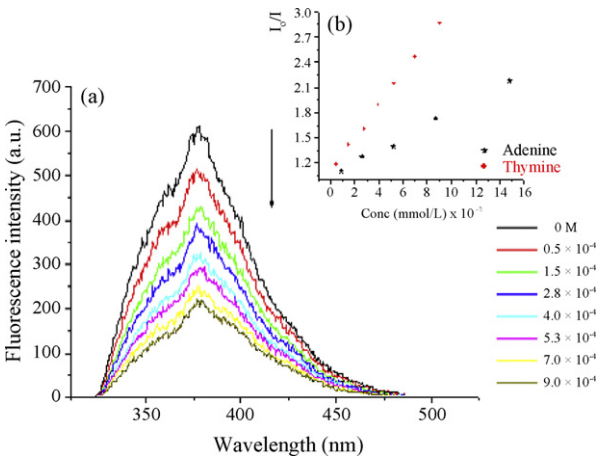

Fig. 6. Fluorescence spectra of AcPheS in DMF solution in the absence and presence of thymine at different concentrations (a) and Stern–Volmer plot for thymine and adenine (b) λ_{ex} = 267 nm.

stilbene fluorescence, and 334 nm characteristic for fluorophore. Therefore, considering the fluorescence spectra of AcPheS recorded in DMF solution (Fig. 5) and excited with 267 nm, there was observed the presence of only monomer fluorescence with a maximum of the emission at 373 nm and two shoulders at 357 nm and 391 nm. In this case, the Stockes shift is about 70 nm, and the absence of the excimer emission provides evidence that there are restrictions concerning the interaction between one excited stilbene unit and another one unexcited to generate excited dimers. Comparatively with the monomer, the PPheS copolymer exhibited a vibronic fluorescence spectrum hypsochromically shifted, possessing a strong fluorescence emission at 358 nm, and four other small shoulders at 340 nm, 376 nm, 398 nm and 432 nm, respectively, as will see later.

Particularly, using the second excitation wavelength (334 nm), in case of monomer it was detected both monomer fluorescence (360 nm) and excimer type (390 nm, 410 nm, 441 nm), promoting the formation of excited dimers. In the same manner, the emission spectra displayed by the copolymer presented a shift to higher wavelengths of the maximum located at 381 nm and other three shoulders at 363 nm, 400 nm and 430 nm. Using DMSO as solvent, a shift towards red with about 30 nm was noticed in the fluorescence maximum of copolymer solution excited at 334 nm, suggesting the appearance of aggregates in the system.

The plot of fluorescence emission for the copolymer in film (not shown) excited with 267 nm, illustrated three larger maxima centered at 360 nm, 390 nm, 420 nm and two small shoulders localized at 447 nm and 485 nm. Modifying the excitation wavelength to 334 nm, for AcPheS the maximum appears at the same wavelength (430 nm) nearby a small shoulder at 455 nm. A comparison between the monomer spectra in solid state with that of PPheS in the film reveals that the maxima are shifted at higher wavelengths (460 nm), while the presence of a small shoulder situated at 520 nm corresponds only to the excimer emission caused of the formation of J-aggregates in the latter.

To gain insight into such short-lived species, we examined the interaction of stilbene fluorophore in solution, in which a quencher is solubilized. Generally speaking, fluorescence quenching determines the diminishing of the quantum yield of fluorescence due to various molecular interactions with quencher molecule, like excited-state reactions, molecules reorganization, energy transfer, ground state complex formation and collision quenching. It is essential to know the type (static or dynamic) and the procedure of quenching in order to establish the mechanism [30]. The fluorescence quenching data are usually evaluated by the Stern–Volmer equation, $I_o/I = 1 + K_{SV}[Q]$, where I and I_o are the fluorescence intensities with or without quencher respectively, $[Q]$ is the quencher concentration and K_{SV} is the Stern–Volmer dynamic quenching constant.

For understanding the quenching mechanism the fluorescence decay profiles of the AcPheS and PPheS solutions in the presence of different purines and pyrimidines type quenchers, like adenine, cytosine, thymine at two different wavelengths, 267 nm and 334 nm, respectively, were measured. Our motivation for choosing the first excitation wavelength is that these bases also present a weak fluorescence at room temperature by which is observable only at specific concentrations [31], the second one being characteristic for fluorophore moiety. Knowing the importance of these nitrogenous bases and the fact that they make up a crucial part of desoxyribonucleotides and ribonucleotides, representing the basis for the universal genetic code, it is clearly why our attention has been paid on this area.

Firstly, the graphical representations of the fluorescence emission in DMF solution of AcPheS using 267 nm excitation wavelength before and after adding adenine (not shown), cytosine (Fig. 5a) and thymine (Fig. 6a) will be discussed. In all three cases, a decrease in fluorescence intensity upon treatment with the mentioned reagents appears. For adenine the detection limit is 0.9×10^{-4} mol/L and for a higher concentration (14.8×10^{-4} mol/L), the fluorescence is reduced with 54% (Fig. 6b). For cytosine, the minimal concentration detected is 0.8×10^{-3} mol/L and the fluorescence intensity is diminished with 68% for 14.4×10^{-3} mol/L. In case of thymine, the detection limit of the quenching agent is 0.5×10^{-4} mol/L, and by adding 9.0×10^{-4} mol/L, the fluorescence intensity is decreased with 65%. Therefore, for the same concentration of purinic or pyrimidinic bases (9.0×10^{-4} mol/L), it can be concluded that the most efficient quencher is thymine, followed by adenine and cytosine. In each case, the quenching process is following Stern–Volmer kinetics, the data fitting on a perfect straight line as it is shown in Figs. 5b, 6b.

Fig. 7a displays plots for quenching of stilbene moiety from PPheS in DMF solution excited with 267 nm at varying concentration of adenine. It was remarked that by adding a quencher solution of 12.2×10^{-5} mol/L adenine, the stilbene fluorescence is reduced with about 63%, the detection limit being 0.8×10^{-5} mol/L. In the presence of 31×10^{-4} mol/L cytosine in the polymer solution, the fluorescence intensity decreases with 72%, and the smallest value of the quencher concentration identified was of 2.8×10^{-4} mol/L (Fig. 8a). Under similar conditions, adding 30×10^{-5} mol/L thymine, the emission of the stilbene sequences from copolymer also showed a decrease of about 74%, the detected minimal concentration being 2.5×10^{-5} mol/L (Fig. 9a). Using the same concentration of quencher (1.22×10^{-4} mol/L), we can summarize that the most efficient is adenine pursued by thymine and cytosine. The plots for

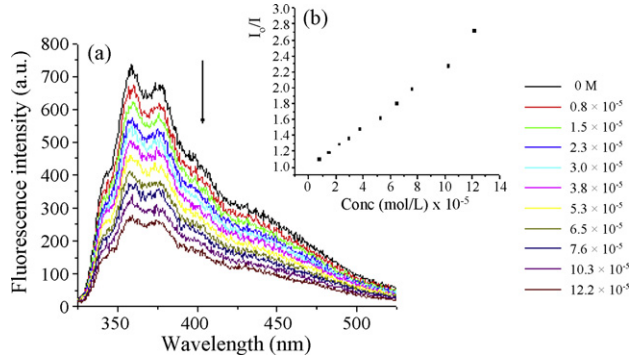

Fig. 7. Fluorescence spectra of PPheS in DMF solution in the absence and presence of adenine at different concentrations (a) and Stern–Volmer plot (b) λ_{ex} = 267 nm.

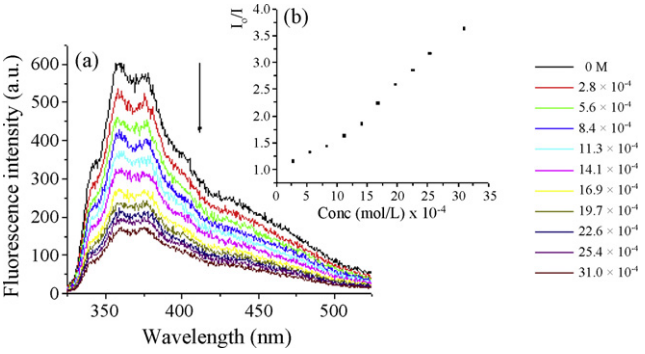

Fig. 8. Fluorescence spectra of PPheS in DMF solution in the absence and presence of cytosine at different concentrations (a) and Stern–Volmer plot (b) λ_{ex} = 267 nm.

I_o/I versus the concentration of added reactant according to the Stern–Volmer equation seems to be linear (Figs. 7b, 8b, 9b). Compared to monomer, the efficiency of polymer quenching by the above bases is over 10-fold increase for adenine, of 4.5 for cytosine and respectively, 3.0-fold for thymine. We, therefore, conclude that the polymer structure is a noticeable advantage in the quenching phenomenon caused mainly by structural factors, while the use of thin films in such experiments would involve another quenchers like volatile amines.

Adjusting the excitation wavelength to 334 nm, the results found for PPheS fluorescence quenching are comparable with those obtained by exciting with 267 nm, adenine being the most efficient reagent. Therefore, by adding 32.9×10^{-4} mol/L of adenine the fluorescence intensity is diminished with 35% and the detection limit was of 0.5×10^{-4} mol/L. On the other hand, a minimal decrease of emission intensity with only 14.7% was observed by adding 139×10^{-4} mol/L of thymine, the limit concentration was of

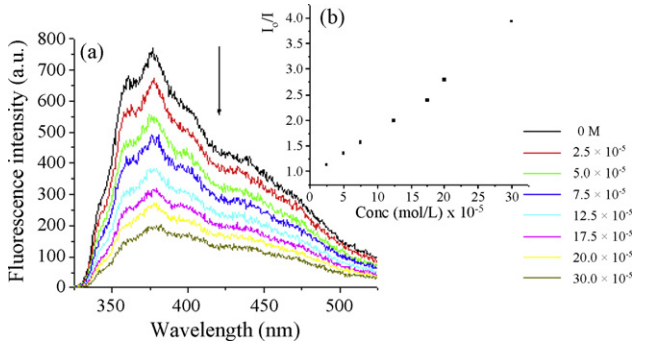

Fig. 9. Fluorescence spectra of PPheS in DMF solution in the absence and presence of thymine at different concentrations (a) and Stern–Volmer plot (b) λ_{ex} = 267 nm.

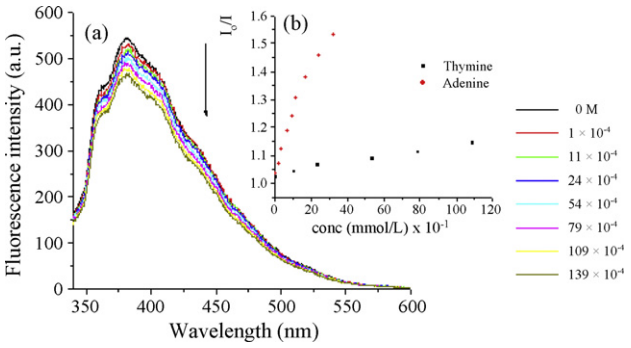

Fig. 10. Fluorescence spectra of PPheS in DMF solution in the absence and presence of thymine at different concentrations (a) and Stern–Volmer plot for thymine and adenine (b) λ_{ex} = 334 nm.

10^{-4} M (Fig. 10a). In the same manner, the Stern–Volmer plots for both quenchers indicated a linear dependence (Fig. 10b). After these observations, it can be remarked the importance of the purinic and pyrimidinic NH_2 and NHCO cation radicals in the discussed system because of an electron transfer which occurs between electron donor (ground state amine) and acceptor molecule (singlet *trans*-stilbene), that yields to an exciplex or an ion pair of type stilbene radical anions and amine radical cations within a solvent cage [26]. Finally, we envisage that the behavior of the stilbene copolymer could be exploited for designing fluorescent sensors for chemical species of interest (purine/pyrimidine bases or aliphatic/aromatic amines) and not only, giving the dual nature of stilbenes which combine two properties, photosensitivity and luminescence in the same functional unit.

4. Conclusions

A novel copolymer, poly((S)-N-acryloylphenylalanine-co-(S)-N-acryloylphenylalanine acid 4-styryl-benzyl ester) (PPheS) was synthesized in order to investigate its photochemical and photophysical properties. It was observed that under UV irradiation the *trans–cis* photoisomerization of the stilbene molecule, accompanied of a [2 + 2] cycloaddition is significantly diminished in a polymeric film owing to local mobility around the chromophore. In addition, the AFM images revealed that the irradiated polymer surface was modified since upon exposure to light the stilbene chromophores change their molecular structure giving rise to conformation changes of the polymeric chains with effect on the physical/chemical properties. The fluorescence study (λ_{ex} = 267 nm, 334 nm) shows that the fluorescence emission of PPheS in DMF solution can be quenched by adenine, thymine, cytosine, the most efficient quencher being adenine. It should be stated that the quenching process occurred via an electron transfer mechanism in which a pyrimidine or purine base participates as an electron donor.

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