VERSITA

Cent. Eur. J. Chem. • 12(10) • 2014 • 1056-1066
DOI: 10.2478/s11532-014-0556-9

Central European Journal of **Chemistry**

Synthesis and characterization of poly [N-acryloyl-(D/L),(+/-)-phenylalanine-co-(D/L),(-/+)N-methacryloyloxyethyl-N'-2-hydroxybutyl(urea)] copolymers

Research Article

Emil C. Buruiana*, Mioara Murariu, Tinca Buruiana

Department of Photochemistry, Petru Poni Institute of Macromolecular Chemistry,
700487 Iasi, Romania

Received 21 October 2013; Accepted 27 January 2014

Abstract: Two monomers of (D/L),(+/-)-N-methacryloyloxyethyl-N'-2-hydroxybutyl(urea) methacrylate (D/L-MABU) type were prepared and further polymerized through free radical polymerization with optically active monomers containing phenylalanine sequences such as N-acryloyl-(D/L),(-/+)-phenylalanine (A-D/L-Phe). The resulting copolymers, i.e., poly[N-acryloyl-(D/L),(-/+)-phenylalanine-*co*-(D/L),(+/-)-N-methacryloyloxyethyl-N'-2-hydroxybutyl(urea)], A-D/L-Phe-*co*-D/L-MABU, were characterized by FT-IR, 1D/2D NMR (^{1}H and ^{13}C), UV-vis, and circular dichroism (CD) spectroscopies, differential scanning calorimetry (DSC), and gel permeation chromatography (GPC). The copolymers obtained with a molar fraction of 0.76 : 0.24 / 0.64 : 0.36 monomer units had optical rotation values of -25° and +15°, respectively. Upon chemical modification of the phenylalanine-based copolymers with fluorescein-isothiocyanate, new fluorescent copolyacrylates (A-D/L-Phe-*co*-D/L-MABU-F) were synthesized and further studied for pH measurements in DMF solutions using HCl and NaOH 10^{-1}M. It was found that sterioselectivity of the A-L-Phe-*co*-L-MABU-F copolymer is higher than of its dextro-form, especially at basic pH.

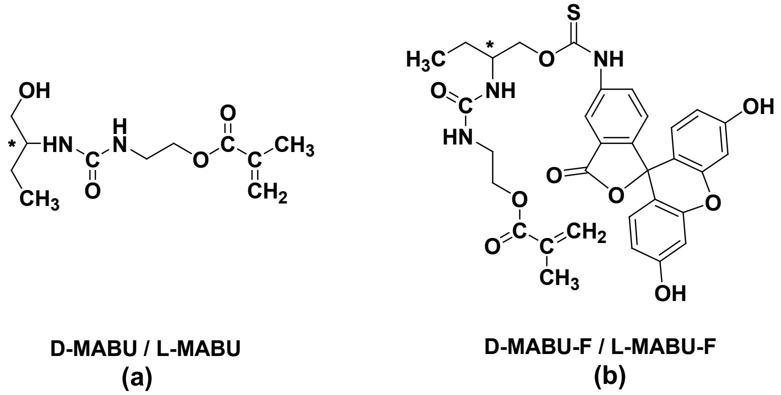
Keywords: *Copolymerization • Polyacrylates • D/L-urea methacrylate • Specific rotation • Fluorescence*

1. Introduction

Synthetic polymers containing chiral α-amino acids either in their side chains or in their main chains have been extensively investigated during the last few decades due to the great importance of the amino acid functionality in increasing the solubility in water and in creating ordered structures through inter- and intrachain association *via* noncovalent forces (hydrogen bonding, dipolar and electrostatic interactions) [1,2]. In fact, the approaches used in the synthesis of such materials led to biopolymers with different topologies, varying from homopolymers to random copolymers, block copolymers and other unique structures. Moreover, by varying the nature of the monomers or their composition and architecture polymers can be suitably tailored for biomedical applications [3-6], including the biosensor area [7] and enantioselective recognition of the D/L amino acids [8,9] among many possible applications. The recent progress in the development of living polymerization techniques such as atom transfer radical polymerization [10] and reversible addition fragmentation chain transfer polymerization [11] allowed for preparation of the polymers of controlled molar mass, narrow polydispersity, and desired chain architecture. Many reports of the use of these methods in polymerization of an optically active monomers based on such amino acids as L-phenylalanine or alanine [12-14], leucine [15], proline [16,17] or valine [18], just to name a few, have already been published. Additionally, it was thought that the introduction of photosensitive groups onto the polymer chain would provide copolymers with chiroptical properties and sensing functionality. For instance, amphiphilic block copolymers with carbazole moieties in their structures and pyrene labelled diblock copolymers with dual

---

* *E-mail: emilbur@icmpp.ro*



Scheme 1. Structures of D-MABU, L-MABU monomers (a) and D-MABU-F, L-MABU-F model compounds functionalized with fluorescein units (b).

temperature and pH-responsiveness were the subjects of some studies because of their potential biomedical applications [19,20]. Also, micelles self-assembled from tryptophan-based block copolymers can serve as fluorescent and chiroptical sensors in either aqueous or organic media [21].

However there have been only a few studies focused on the synthesis of chiral polymers obtained *via* conventional free radical polymerization of the vinyl monomers carrying amino acids like proline, L-valine, L-leucine or phenylalanine [22-25]. Some of these polymers, especially the methacryloyl/acryloyl derivatives of glycine, L-glutamic acid, and alanine have been used in production of dental materials [26]. In the series of photoluminescent copolymers, our group published the synthesis of polymers with phenylalanine and different fluorescent groups supported by a fluorescence study [27,28].

Considering all the factors, in this study we present the preparation of optically active monomers (D/L),(+/-)-N-methacryloyloxyethyl-N'-2-hydroxybutyl (urea), (D/L-MABU), which are further subjected to free radically copolymerization with N-acryloyl-(D/L),(-/+)-phenylalanine (Ac-D/L-Phe), to produce the copolymers with amino acid and urea groups in their side chains. Subsequently, the synthesized copolymers were functionalized with the fluorescein isothiocyanate isomer I in order to obtain optically active and fluorescent photopolymers, appropriate for chemosensor applications in biology, biomedicine, analytic techniques, and optical devices.

2. Experimental procedure

2.1. Materials

(D/L),+/-)-phenylalanine, acryloyl chloride, (D/L),(-/+)-2-amino-1-butanol, 2-isocyanatoethyl methacrylate, fluorescein isothiocyanate isomer I, dibutyltin dilaurate, azobis(cyclohexanecarbonitrile), and all the solvents were purchased from Aldrich and were used without further purification.

2.2. Monomers synthesis

N-acryloyl-(D/L),(-/+)-phenylalanine vinyl monomers**,** A-D-Phe and A-L-Phe**,** were prepared in a basic medium under the conditions similar to the conditions reported in literature [27].

FT-IR A-D/L-Phe (cm^{-1}): 1712 (COOH), 1651 (amide I), 1596 and 940 (–C=C–), 1536 (amide II). ^{1}H-NMR spectrum of A-D-Phe monomer in DMSO-d$_6$ (*δ*, ppm): 12.7 (1H, s, -COO$\underline{H}$), 8.45 (1H, d, N$\underline{H}$), 7.22 (5H, m, $C_6\underline{H}_5$), 6.28 (1H, m, trans $C\underline{H}_2$=CH), 6.07 (1H, d, CH_2=C$\underline{H}$CO), 5.59 (1H, d, cis $C\underline{H}_2$=CH), 4.5 (1H, m, C$\underline{H}$–COOH), 3.15 – 2.8 (2H, m, $C\underline{H}_2$-C_6H_5).

(D/L),(+/-)-N-methacryloyloxyethyl-N'-2-hydroxybutyl(urea) monomers, D-MABU and L-MABU, (Scheme 1a) were prepared by dropwise addition of 2-isocyanatoethyl methacrylate (4.35 g, 27.48 mmol) to (D/L),(-/+)-2-amino-1-butanol (2.5 g, 27.48 mmol) dissolved in 10 mL of tetrahydrofuran. The reaction was performed at 25°C for 24 h and the precipitate formed was collected on a filter and dried. Yield: 5.6 g (81.75%).

Synthesis of *(D/L)-N-methacryloyloxyethyl-N'2-fluoresceinylthiourethane-butyl(urea)* (D-MABU-F and L-MABU-F)

The fluorescent monomers (Scheme 1b) were obtained using 0.4 g (1.637 mmol) (D/L)-N-methacryloyloxyethyl-N'-2-hydroxybutyl(urea) and a stoichiometric amount of fluorescein isothiocyanate isomer I (0.637 g, 1.637 mmol) in THF in the presence of dibutyltin dilaurate (DBTD) catalyst. The well-stirred mixture was heated at 55ºC under reflux for 24 h. The resulting monomers, D-MABU-F and L-MABU-F, were precipitated in water and collected by filtration, and vacuum-dried. Yield: 0.964 g (93%).

2.3. Polymers synthesis

Synthesis of *poly[N-acryloyl-(D/L),(-/+)-phenylalanine-co-(D/L),(+/-)-N-methacryloyloxyethyl-N'-2-hydroxybutyl (urea)]*, A-D-Phe-*co*-D-MABU and A-L-Phe-*co*-L-MABU

Synthesis of A-D/L-Phe-*co*-D/L-MABU was carried out through a conventional radical polymerization using the following procedure: in each of the two polymerization ampoules equipped with a magnetic stirrer, 3 g (13.698 mmol) of A-D/L-Phe was dissolved in 25 mL of dimethylformamide (DMF). Then, 5.2 mg (1 wt‰) of azobis(cyclohexanecarbonitrile) (ABCN) was dissolved in 4 mL of DMF, and this solution was divided equally into two parts, each being added to the two polymerization ampoules containing the monomer solution of A-D/L–Phe. In other two flasks, 0.6 g (2.448 mmol) of either D-MABU or L-MABU sample was dissolved in 5 mL of DMF and then, each of them was transferred into the two polymerization ampoules as follows: the D-MABU monomer solution was added to the A-D-Phe solution and the L-MABU solution was added to the A-L-Phe monomer solution. Both polymerization reactions were carried out using molar fractions of A-D/L-Phe: D/L-MABU monomers of 0.76 : 0.24 and 0.64 : 0.36, under purified nitrogen at a temperature of 80°C for 3 days. The resulting compounds were precipitated in distilled water, filtered, and washed several times. Finally, the formed copolymers were dried under reduced pressure at 60°C for 24 h. Yield: 2.8 g (80%).

Synthesis of *poly[N-acryloyl-(D/L),(+/-)-phenylalanine-co-(D/L)-N-methacryloyloxyethyl- N'2-fluoresceinylthiourethane-butyl(urea)]*, A-D-Phe-*co*-D-MABU-F and A-L-Phe-*co*-L-MABU-F

Functionalization of all the copolymers with fluorescence units was performed under similar conditions which were as follows: 1 g of A-L-Phe-*co*-L-MABU copolymer was dissolved in 10 mL DMF and then 0.085 g (0.2185 mmol) of fluorescein isothiocynate isomer I and a catalytic amount of DBTD were added. The mixture was stirred at 56°C for 24 h, and the final product (A-L-Phe-*co*-L-MABU-F) was precipitated into diethyl ether, filtered, and dried at 60°C, for 24 h, under reduced pressure. Yield: 0.6 g (55.3%).

2.4. Measurements

Proton nuclear magnetic resonance spectroscopy (^{1}H-NMR) spectra were recorded on a Bruker Advance III 400-MHz spectrometer in either $CDCl_3$ or DMSO-d_6 at room temperature. ^{1}H-NMR and ^{13}C-NMR chemical shifts were given in δ units (ppm) as compared to the residual peak of the solvent (^{1}H-NMR: DMSO, 2.51 ppm; $CDCl_3$: 7.26 ppm); ^{13}C-NMR: DMSO, 39.47 ppm; $CDCl_3$: 77.2 ppm). 2D ^{1}H,^{1}H-COSY (Correlation Spectroscopy) and ^{13}C,^{1}H-HSQC (Heteronuclear Single Quantum Coherence) studies were performed using standard pulse sequences in the version with z-gradients, as supplied by Bruker with TopSpin 2.1 PL6 operating software. Fourier Transform infrared (FT-IR) spectra and UV absorption spectra were recorded on a Bruker Vertex 70 spectrometer and a Specord 200 spectrophotometer respectively, in DMF. The fluorescent spectra were recorded using a Perkin–Elmer LS55 spectrophotometer, at room temperature, in a DMF solution. The average molecular weights were determined in DMF by gel permeation chromatography (GPC) measurements consisting of a model Polymer Laboratory EMD-950 apparatus equipped with an evaporative mass detector and two PL gel 5 µm columns. The samples were of 2.0 g dL^{-1} polymer solution in *N,N*-dimethylformamide with 0.1 M LiCl and the flow rate of the carrier solvent was 1 mL min^{-1}. The average molecular weight was calculated on the basis of the molecular weight *versus* retention volume curve of monodisperse polystyrene (PS) standard with a molecular range from 580 to 3150 kD. Thermal transitions were measured through the Differential Scanning Calorimetry (DSC) in a nitrogen atmosphere on a STA 449 ***F1 Jupiter*** (Netzsch, Germany) at a heating rate of 10°C min^{-1}. CD spectra were recorded using a ChirascanTM CD Spectrometer from Apllied Photophysics. All optical rotation measurements were carried out in distilled water (c = 100 µM) at 25.0 ± 0.2°C using quartz cells with a path length of 2.0 mm. Data were collected at the wavelengths from 180 to 260 nm in 1.0 nm increments. Optical activity experiments were performed in a 0.1 N sodium hydroxide solution (c = 0.01 g cm^{-3}) at 23°C on an OPTIK Pol 1 polarimeter with the cell path length of 1 dm (λ = 590 nm).

3. Results and discussion

3.1. Synthesis and characterization

Copolymers with D/L,(-/+)-phenylalanine and D/L,(+/-)-butanol(urea) moieties (A-D-Phe-*co*-D-MABU and A-L-PheAla-*co*-L-MABU) in their structures were synthesized through a conventional free radical polymerization of (D/L),(+/-)-N-methacryloyloxyethyl-N'-2-hydroxybutyl(urea), (D/L-MABU) with N-acryloyl-(D/L),(-/+)-phenylalanine, (A-D/L-Phe). In the first step, the parent monomers, D/L-MABU, (Scheme 1a) were obtained through the addition reaction of methacryloyloxyethyl-2-isocyanate to D/L, (-/+)-2-amino-1-butanol or *via* a Schotten–Baumann

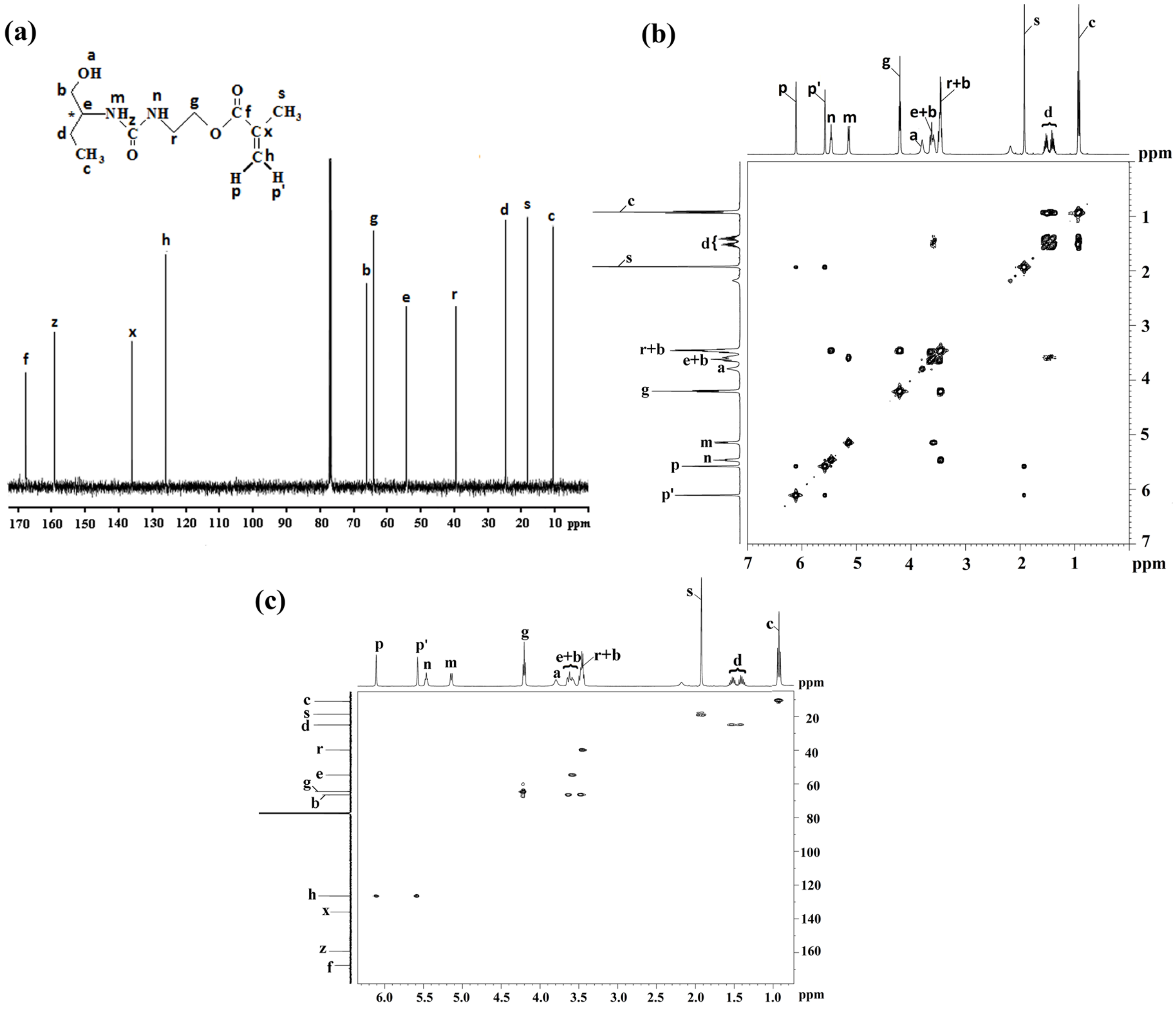


Figure 1. ^{13}C-NMR (a), ^{1}H,^{1}H-COSY (b) and ^{13}C,^{1}H-HSQC (c) spectra of the L-MABU monomer in CDCl$_3$.

reaction using acryloyl chloride as an acylation agent reacting with D/L,(+/-)-phenylalanine. The structures of both monomers were confirmed through spectral analysis (1D ^{1}H-NMR, 1D ^{13}C-NMR, 2D ^{1}H,^{1}H-COSY, 2D ^{13}C,^{1}H-HSQC, and FT-IR). Fig. 1 presents the ^{13}C-NMR (a), ^{1}H,^{1}H-COSY (b) and ^{13}C,^{1}H-HSQC (c) spectra of L-MABU monomer containing the urea groups, in CDCl$_3$, where the characteristic peaks of the constituent protons were identified as follows: the methacrylate protons in *trans*- and *cis*- configuration appeared at 6.12 and 5.62 ppm, respectively, while the urea protons exhibited peaks at 5.49 and 5.17 ppm, respectively. The methylene protons of the ester groups have been observed as singlet peaks at 4.25 ppm, while the methylene protons attached to the hydroxyl function give the signals located at 3.66 and 3.50 ppm, respectively. The resonance peaks at 3.66 and 3.50 ppm are overlapping with the methine and methylene protons near the urea group. Other specific signals are positioned at 3.83 ppm (hydroxyl proton), 1.96 ppm (methyl protons of methacrylate), 1.62 - 1.37 ppm (methylene protons in an α position to the CH group), 0.96 ppm (methyl protons in the β position to the CH group). Further confirmation of L-MABU chemical structure is supported by the ^{13}C-NMR spectrum (Fig. 1b) where the chemical shifts of the characteristic signals belong to the carbonyl of the ester group (167.65 ppm), the carbonyl of urea (159.27 ppm), the olefinic carbons of methacrylate (136.00 and 126.08 ppm), the methylene carbon near the hydroxyl group (66.07 ppm) and the ester function (64.04 ppm). The signals of themethine and methylene carbon near the urea group (54.34 and 39.48 ppm), the methyl carbon of methacrylate (18.23 ppm), and the methylene (24.63 ppm) and the methyl (10.60 ppm) groups of the urea derivative are also seen in the spectrum. The assignments of the ^{13}C-NMR chemical shifts were

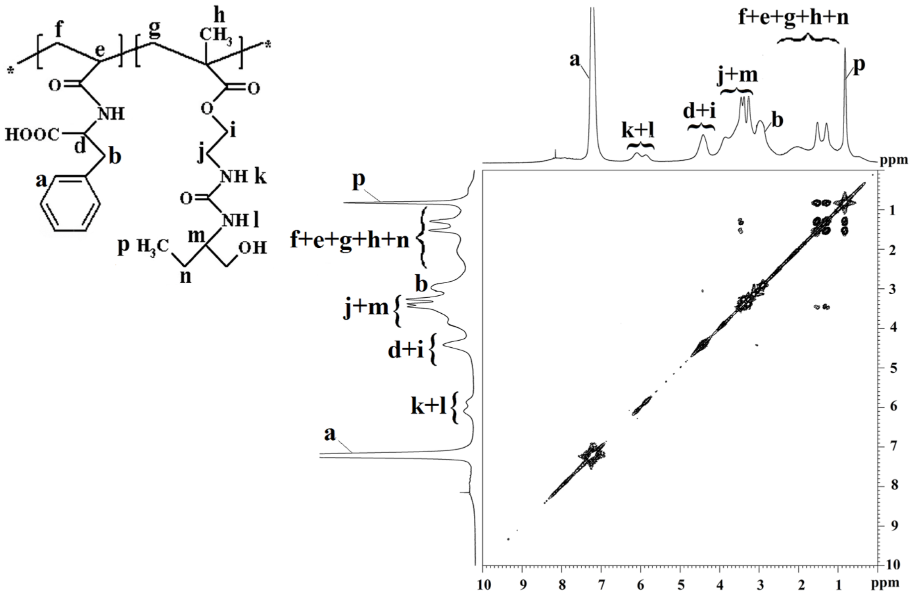


Figure 2. ^{1}H,^{1}H-COSY spectrum of A-L-Phe-*co*-L-MABU copolymer, in DMSO-d_6.

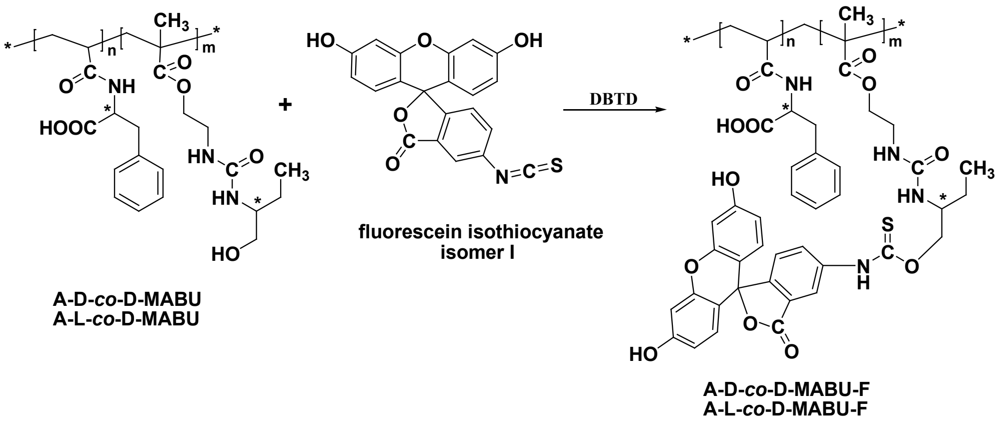
carried according to the numbering scheme shown in Fig. 1a. The *J*-coupled protons were assigned from the ^{1}H,^{1}H-COSY spectrum presented in Fig. 1b, while the assignments of the carbons of the CH groups were based on the ^{13}C, ^{1}H-HSQC spectrum shown in Fig. 1c.

The FT-IR spectrum of D-MABU (not shown here) lacks the characteristic absorption band of the NCO group (2270 cm^{-1}), thus indicating the involvement of isocyanate moiety in the reaction with NH_2 groups. The absorption band centered at 3321 cm^{-1} is ascribed to the stretching vibration of the OH or NH groups, while the peak around 1631 cm^{-1} corresponds to the amide I and C=C groups, and those at 1542 and 1460 cm^{-1} to the amide II and amide III vibrations. The inflexions at 1178 and 817 cm^{-1} are characteristic of the carbonyl ester (CO-O) and carbon–carbon double bonds (C=C), respectively.

Upon classical copolymerization reactions of A-D/L-Phe with D/L-MABU**,** new copolymers with phenylalanine and urea moieties, poly[N-acryloyl-(D/L),(-/+)-phenylalanine-*co*-(D/L),(+/-)-N-methacryloyloxyethyl-N'-2-hydroxybutyl(urea)], abbreviated A-D-Phe-*co*-D-MABU and A-L-Phe-*co*-L-MABU were obtained. The structures of these copolymers were confirmed by the 2D NMR study, ^{1}H,^{1}H-COSY. As compared to the L-MABU, the ^{1}H,^{1}H-COSY spectrum of the A-L-Phe-*co*-L-MABU copolymer (Fig. 2) displays resonance signals at 2.4 – 0.46 ppm corresponding to the methine, methylene and methyl protons from the polymer main chain and the methylene protons linked to the ester group of L-MABU. The methylene protons of A-Phe appear as a multiplet in the region of 3.3 - 3.1 ppm, and the methine proton connected to the carboxyl and the methylene protons attached to the ester group can be identified at 4.5 - 4.2 ppm. Using the integrals values corresponding to the protons signals of the phenyl group (7.22 ppm) *vs.* methyl protons (0.82 ppm) from the urea monomer, the molar fraction of the monomer units in the synthesized A-L-Phe-*co*-L-MABU copolymer was calculated as 0.64 : 0.36 (A-L-Phe : L-MABU).

The FT-IR spectrum of the A-L-Phe-*co*-L-MABU copolymer (not shown) revealed the presence of the absorption bands centered at 3385 cm^{-1} and 1727 cm^{-1} attributed to the stretching vibration of the OH/NH and the carbonyl groups, respectively. Additionally, the absorption peak at 1210 cm^{-1} could be associated with the carbonyl ester, and the absence of the carbon–carbon double bonds (C=C) absorption in the infrared spectrum of the copolymer indicated the complete polymerization of the above mentioned monomers. The molecular weights of the copolymers were evaluated in a DMF solution from GPC *via* PS standard. The GPC measurements of A-D-Phe-*co*-D-MABU and A-L-Phe-*co*-L-MABU gave the molecular weights of 8170 (M_w/M_n = 1.47) and 9650 (M_w/M_n = 1.45), respectively.

Since the physical properties of copolymers depend on the glass transition temperature (T_g), the thermal behavior of our copolymers was examined by DSC analysis. The DSC thermograms recorded for A-D-Phe-*co*-D-MABU and A-L-Phe-*co*-L-MABU proved the existence of a single glass transition (T_g) at about 113.24°C and 118.5°C, respectively, that confirms the homogeneity and purity of the synthesized copolymers. Moreover, the higher T_g values for these copolymers



Scheme 2. Synthesis of copolymers functionalized with fluorescein units A-D-Phe-co-D-MABU-F and A-L-Phe-co-L-MABU-F.

can be correlated with a higher rigidity of their polymeric backbone.

The specific optical rotation measurements for D-MABU and L-MABU in a 0.1N NaOH solution revealed values of $[\alpha]_D^{23}$ = +20° and $[\alpha]_D^{23}$ = -35°, but the direction of rotation is different compared to that of the amino acid monomers (A-D-Phe, $[\alpha]_D^{23}$ = -80° and A-L-Phe, $[\alpha]_D^{23}$ = +60°). In the case of A-D-Phe-*co*-D-MABU and A-L-Phe-*co*-L-MABU copolymers, optical rotation values of -25° and +15° were obtained and these results are being correlated to the higher rotary power of the monomer containing the amino acid. A comparison between the optical rotation values of the synthetic molar mixture based on A-D-Phe (0.76): D-MABU (0.24), namely -50° or of +35° for A-L-Phe (0.64) : L-MABU (0.36), and the synthesized copolymers indicated a considerable decrease in the absolute value of the polymers optical activity that arose from the principal chain conformations.

Further, the above copolymers (A-D-Phe-*co*-D-MABU and A-L-Phe-*co*-L-MABU) were reacted with the fluorescein isothiocyanate isomer I in the presence of DBTD to obtain photopolymers with fluorescent properties (A-D-Phe-*co*-D-MABU-F and A-L-Phe-*co*-L-MABU-F) (Scheme 2), whose structures were confirmed by ^{1}H-NMR and FT-IR spectroscopies. The ^{1}H-NMR spectrum of the A-L-Phe-*co*-L-MABU-F copolymer (Fig. 3) showed the appearance of resonance signals attributed to the specific functional groups, similar to those belonging to A-L-Phe-*co*-L-MABU, with exception of the fluorescein protons signals in the 6.75 - 6.45 ppm region. From the integral ratio of the fluorescein protons and the methyl protons (0.82 ppm) the functionalization degree of the above copolymers was estimated to be about 1%. The determination of the optical rotation for A-D-Phe-*co*-D-MABU-F and A-L-Phe-*co*-L-MABU-F indicated the values of $[\alpha]_D^{23}$ = -45° and $[\alpha]_D^{23}$ = +10°, respectively. Therefore, a post-chemical modification of the polymers that occured at the α-position of the asymmetric carbon atom caused the changes in the photopolymers rotation sign only in the case of D-isomer.

To prove the functionalization of the A-L-Phe-*co*-L-MABU and A-D-Phe-*co*-D-MABU-copolymers with fluorescent molecules, two model compounds, D-MABU-F and L-MABU-F (given in Scheme 1), were prepared through the addition reaction of D-MABU and L-MABU monomers with the fluorescein isothiocyanate isomer I. The chemical structures of these compounds were confirmed by FT-IR, ^{1}H-NMR, and UV spectroscopies. In the ^{1}H-NMR spectrum of the L-MABU-F monomer (not shown) the proton signals of the fluorescein are positioned in the region of 7.5 – 6.5 ppm, while the methylene protons from the thiourethane ester appear at 4.06 ppm. Other specific signals are similar to those of L-MABU. In the FT-IR spectrum of L-MABU-F, the absorption bands characteristic of the isothiocyanate (NCS) groups (2070 cm^{-1}) disappeared, thus confirming the complete reaction.

3.2. Circular dichroism study

The enantiomers are non *superimposable* mirror images, and are considered to be chemically identical. The helix and random coil configurations of the A-D-Phe, A-L-Phe, D-MABU, L-MABU monomers and the A-D-Phe-*co*-D-MABU, A-L-Phe-*co*-L-MABU copolymers, as well as their transitions, were determined by the circular dichroism spectroscopy (CD) in water. These measurements refer to the differential absorption of the left-handed (counter-clockwise, L) polarized light against the right-handed (clockwise, D) polarized light which occurs due to the structural asymmetry of a substance under the same conditions. Fig. 4 shows the CD spectra of the A-D-Phe-*co*-D-MABU and A-L-Phe-*co*-L-MABU

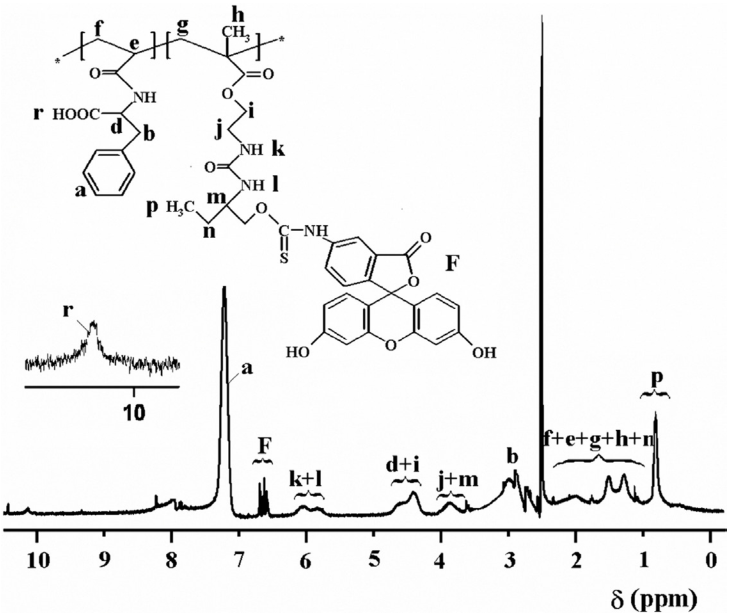


Figure 3. ^{1}H-NMR spectrum of the A-L-Phe-*co*-L-MABU-F copolymer functionalized with fluorescein units, in DMSO-d_6.

copolymers, which adopt the ordered conformations similar to their theoretical helix shape. As expected, the enantiomers exhibit the mirroring effect, namely the dichroic absorption of almost the same magnitude but of opposite sign, confirming the correctness of the obtained data. The CD spectrum of A-D-Phe-*co*-D-MABU reflects the helix formation in water, with an intense positive band (π - $\pi^*_{perpendicular}$ transition) blue-shifted at 187 nm, the first negative band (π - $\pi^*_{parallel}$ transition) blue-shifted at 199 nm, and the second negative band (n- π^* transition) shifted toward lower wavelength (~215 nm). Similarly, the CD mirror image spectrum of A-L-Phe-*co*-L-MABU is characterized by the presence of a negative band (π - π^* transition) below 200 nm, and a two less intensive positive bands between 198 - 220 nm.

The two enantiomers of the A-D-Phe and A-L-Phe monomers behaved in the same way as the aforementioned copolymers, their CD spectra (Fig. 5) presenting the mirror images of the right handed and left handed α-helices, respectively. A-D-Phe showed an ordered conformation characterized by an intensive positive band blue-shifted at 188 nm and two broad negative bands blue-shifted at 200 and 217 nm as a proof of the presence in the synthesized copolymers of the monomers containing phenylalanine induced helix conformation. The only noticeable difference between the two spectra was the appearance of two shoulders in the curve of the spectrum A-D-PheAla, located at 212 and 232 nm, probably due to the remaining unreacted precursors.

The CD spectra of both monomers containing the urea groups, recorded in water, suggested the predominance of a random coil conformation (not shown). The D-MABU monomer showed a positive band at 189 nm and three negative CD bands at 208, 220 and 226 nm. The CD spectrum of the second enantiomer, L-MABU, sustains that the dichroic absorption does not take into consideration the conformation of the previous bands with opposite sign, namely five positive bands centered at 184, 195, 208, 220, and 230 nm, and a negative one at 188 nm.

3.3. Fluorescence properties

Fluorescein dyes are one of the most widely used fluorescent probes, one of their representative derivatives being isothiocyanate, a compound that can be covalently attached to amino acids and to macromolecules. The absorption and emission properties of the A-D-Phe-*co*-D-MABU-F and A-L-Phe-*co*-L-MABU-F fluorescent copolymers and of their corresponding model compounds (D-MABU-F, L-MABU-F) are influenced by the pH of the local environment and any change in pH can strongly affect their fluorescence characteristics [29]. The photophysical properties of these products were studied using UV-vis absorption and photoluminescent spectroscopy. In case of the D-MABU-F and L-MABU-F model compounds, the UV–vis absorption spectra (not shown) contained three absorption bands located at 279, 289 and 446 nm which could indicate the presence of the fluorescein species such as lactone (around 446 nm) or a cationic moiety. The UV–vis absorption

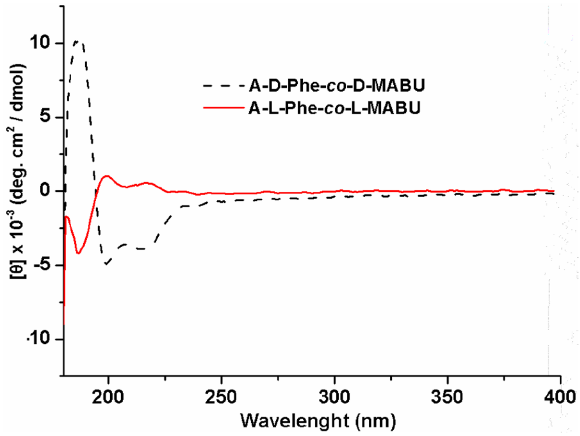


Figure 4. Circular dichroism spectra of copolymers A-D-Phe-*co*-D-MABU (- - -) and A-L-Phe-co-L-MABU (——) in distilled water, in various conformations.

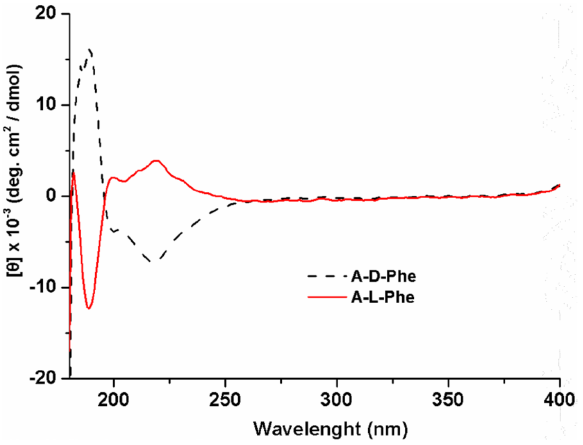
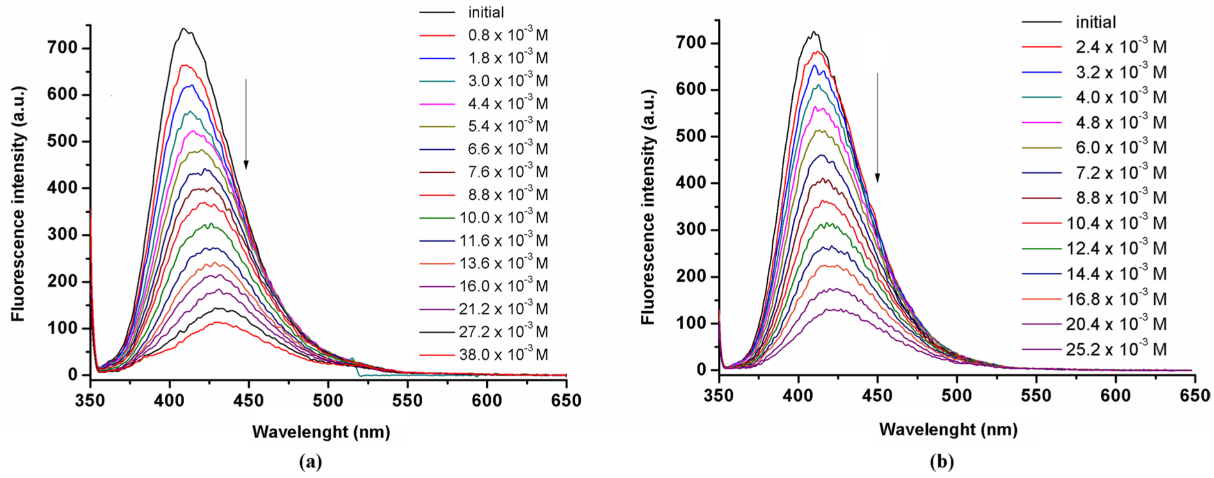


Figure 5. Circular dichroism spectra of monomers A-D-Phe (- - -) and A-L-Phe (——) in distilled water, in various conformations.

spectra of the A-D-Phe-*co*-D-MABU-F and A-L-Phe-*co*-L-MABU-F copolymers showed two distinct absorption maxima around 278 nm and a small shoulder at 289 nm accompanying the main peak, which suggested the existence of only one species, *i.e.*, the cationic form. The emission spectra of the fluorescein-labeled acrylic copolymers and the aforesaid model compounds were recorded in dilute solutions of DMF by using an excitation wavelength λ_{ex} = 340 nm. The D-MABU-F and L-MABU-F model compounds exhibited the emission maximum at around 406 nm, while in the case of the A-D-PheAla-*co*-D-MABU-F and A-L-PheAla-*co*-L-MABU-F copolymers these maxima appeared at 412 nm; in both structures this effect was related to the monomer fluorescence. The internal motion of macromolecules was emphasized by the fluorescence quenching of fluorescein molecules using using different volumes of HCl and NaOH 10^{-1} M as quenching agents. As a result, a gradual decrease of the emission intensity of A-D-Phe-*co*-D-MABU-F (Fig. 6a) and A-L-Phe-*co*-L-MABU-F (Fig. 6b), with 85% and 82%, respectively, through the addition of final concentrations of HCl solutions (38×10^{-3} mol L^{-1} and 25.2×10^{-3} mol L^{-1}) has been observed. This quenching process can be attributed to the acid diffusion within the proximity of fluorescein molecules, leading to the deactivation of the singlet excited state. Upon protonation in acidic medium, the opening of the lactone ring of fluorescein molecules followed by the bathochromic shift of the emission maximum can be registered, the detection limit being of 0.8×10^{-3} mol L^{-1} for A-D-Phe-*co*-D-MABU-F and of 2.4×10^{-3} mol L^{-1} for A-L-Phe-*co*-L-MABU-F.

Fig. 7 depicts the emission spectra of A-D-Phe-*co*-D-MABU-F and A-L-Phe-*co*-L-MABU-F in the presence of different volumes of NaOH 10^{-1} M, where



(a)

(b)

Figure 6. Fluorescence spectra of A-D-Phe-co-D-MABU-F (plot a) and A-L-Phe-co-L-MABU-F (plot b) in DMF solution, in the absence and the presence of HCl 10^{-1}M at different concentrations; λ_{ex} = 340 nm.

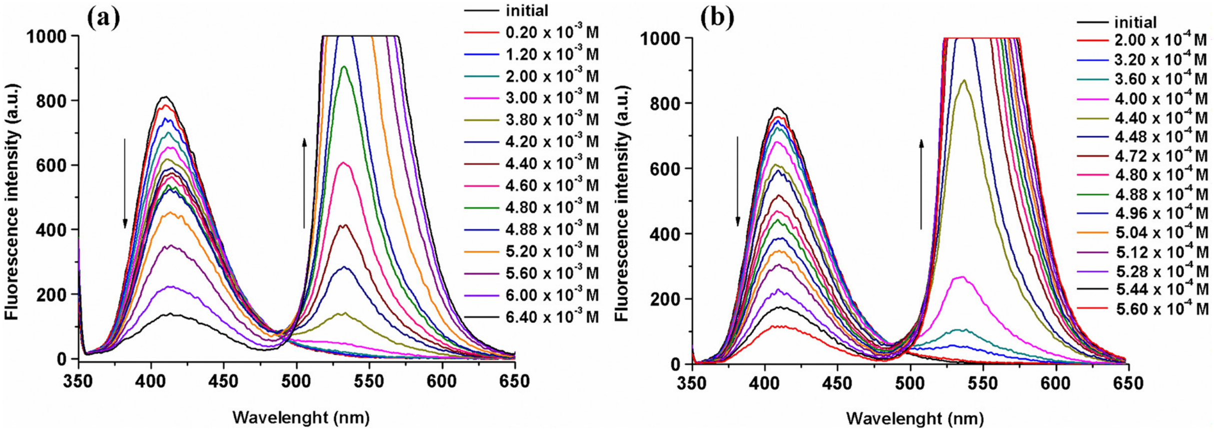


Figure 7. Fluorescence spectra of A-D-Phe-*co*-D-MABU-F (plot a) and A-L-Phe-*co*-L-MABU-F (plot b) in DMF solution, in the absence and the presence of NaOH at different concentrations; λ_{ex} = 340 nm.

it can be seen that the monomer fluorescence intensity decreases as the concentration of NaOH increases, and the detection limit for both copolymers was 2×10^{-4} mol L^{-1}. At higher quencher concentration (6.4×10^{-3} mol L^{-1} NaOH for A-D-Phe-*co*-D-MABU-F and 5.6×10^{-3} mol L^{-1} for A-L-Phe-*co*-L-MABU-F) the fluorescence emission is quenched by around 82% and 85%, respectively. The same effect was recorded in the fluorescence spectra of their model compounds, where the fluorescence emission intensity decreased by about 93% and 87%, when NaOH concentration reached 6.08×10^{-4} mol L^{-1} and 1.20×10^{-4} mol L^{-1}, respectively. In the latter case, the detection limit was 4×10^{-5} mol L^{-1} for D-MABU-F and 8×10^{-6} mol L^{-1} for L-MABU-F. Hence, with increasing basicity of the solution the opening of the lactone ring with the formation of both the quinoid and dianion (containing COO^{-} and phenoxide ions) structures can be taken into account for all synthesized compounds [30,31]. These remarks were supported by the appearance of new absorption peaks in the corresponding UV-vis spectra (not shown) recorded after addition of the quencher (313, 484 and 518 nm in D-MABU-F and L-MABU-F, as well as in A-D-Phe-*co*-D-MABU-F and A-L-Phe-*co*-L-MABU-F copolymers (307, 480 and 511 nm). Moreover, the shift of the emission maximum from 406 nm (correlated with the opening of lactone ring) and the appearance of a new emission maximum at 535 nm (formation of the overlapped quinoid and dianion structures) can be evident for all compounds. Although the fluorescence quenching for both A-D-Phe-*co*-D-MABU-F and A-L-Phe-*co*-L-MABU-F copolymers was similar (about 80 %), the quantity of NaOH 10^{-1} M used for A-L-Phe-*co*-L-MABU-F was less than the one used for A-D-Phe-*co*-D-MABU-F, therefore one can conclude that A-L-Phe-*co*-L-MABU-F is more sensitive to the pH variations of basic solutions.

The efficiency of fluorescence quenching of thiofluorescein moiety of such compounds, in DMF solution, was evaluated using Stern-Volmer equation. The plots are obtained by a graphical representation of the ratio of fluorescence intensities in the absence and presence of a quencher (I_0/I), towards the concentration of the added quencher [Q], according to the Stern-Volmer equation: $Io/I = 1 + K_{SV}[Q]$, where Ksv is the Stern-Volmer quenching constant. In the presence of HCl 10^{-1} M solution (Fig. 8, plot a), we can observe that the plots for Io/I appear to be linear over the concentration range investigated for all the fluorescent models and the polymers used in this study (kinetics of first order), suggesting the involvement of only one quenching mechanism, whereas the use of NaOH 10^{-1} M (Fig. 8, plots b and c) solution resulted in a deviation from linearity, thus indicating the co-existence of two competitive processes. Perhaps the most interesting result is that the enantioselectivity of the A-L-Phe-*co*-L-MABU-F copolymer is higher (10-fold increase in magnitude) than that of A-D-Phe-*co*-D-MABU-F and this amplification of the fluorescence intensity (535 nm) in the former should be used to discriminate the chirality of the resulting polymers.

4. Conclusions

Poly[N-acryloyl-(D/L),(-/+)-phenylalanine-co-(D/L),(+/-)-N-methacryloyloxyethyl-N'-2-hydroxybutyl (urea)] were prepared by free radical polymerization using new optically active monomers: (D/L),(+/-)-N-methacryloyloxyethyl-N'-2-Hydroxybutyl(urea) and N-acryloyl-(D/L),(-/+)-phenylalanine. These copolymers showed characteristic chiroptical properties, their CD spectra suggesting the helix formation in an aqueous

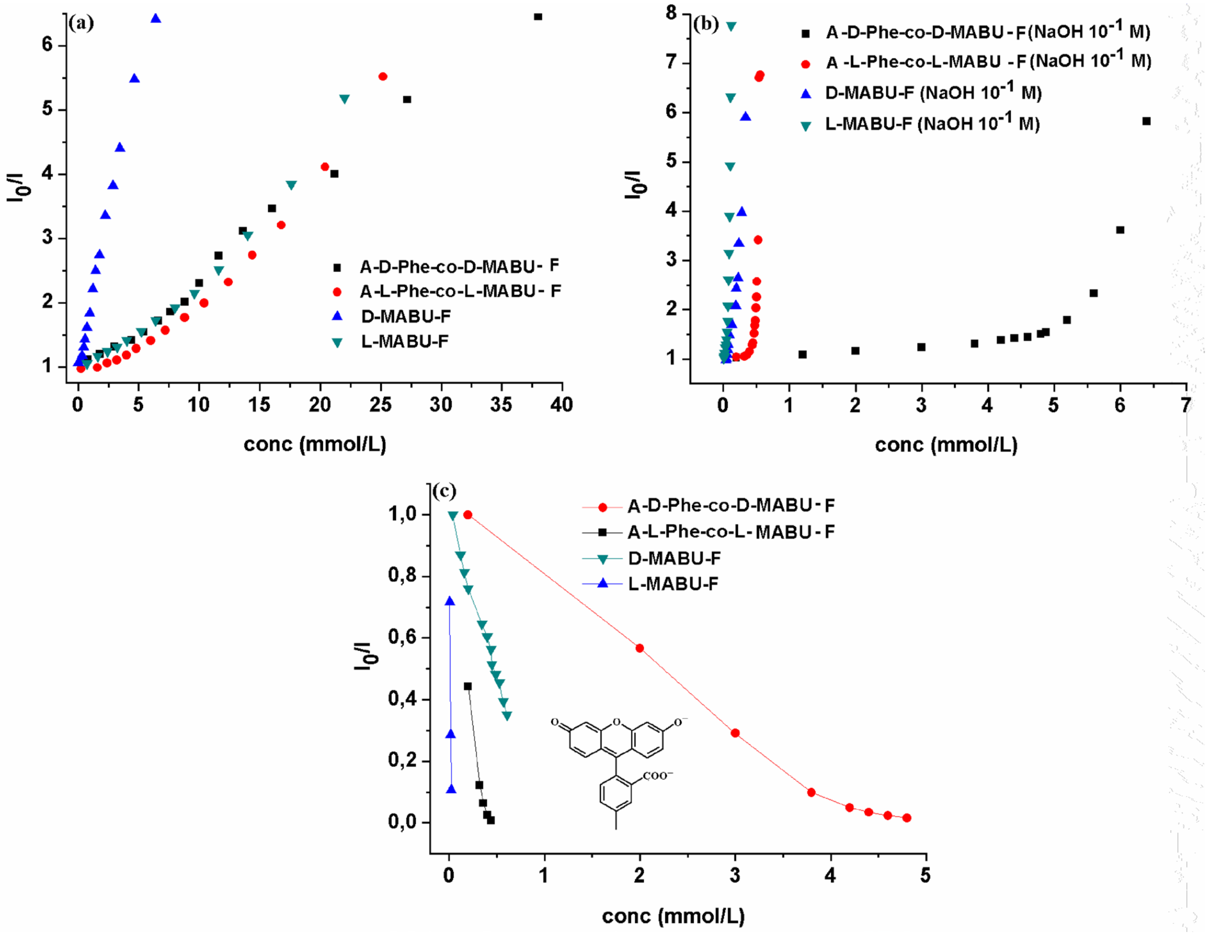


Figure 8. Stern–Volmer plots for copolymers A-D-Phe-co-D-MABU-F and A-L-Phe-co-L-MABU-F, and their model compounds in presence of HCl 10^{-1}M (a) and NaOH 10^{-1}M (b) - the first maxim from 406 and 409 nm for monomers (D-, L-), and at 409 for (D-, L-) copolymers, and (c) - the second maxim from 537 nm for monomers (D-, L-,) and at 532 and 535 nm for (D-, L-) copolymers.

solution, with three positive/negative bands at 187, 199, and ~215 nm for poly[N-acryloyl-D-phenylalanine-co-D-N-methacryloyloxyethyl-N’-2-hydroxybutyl (urea)], and below 200 nm, and between 198 - 220 nm, in the later (L-copolymer). Upon further chemical functionalization with 1% fluorescein moieties, the specific rotation values of the fluorescein-labeled copolymers increased for the D-isomer comparatively with the non-modified copolymer. The fluorescence data collected after adding NaOH 10^{-1} M solutions showed that the fluorescence intensity at 535 nm increased for both copolymers, but a more significant increase was found in the case of L-copolymer (4.4×10^{-4} mol L^{-1}) than the D-isomer (2.0×10^{-3} mol L^{-1}). These results demonstrate a good selectivity of the fluorescent L-copolymer in chiral recognition of the urea fluorescein derivatives.

References

[1] E.G. Bellomo, M.D. Wyrsta, L. Pakstis, D.J. Pochan, T.J. Deming, Nat. Mater. 3, 244 (2004)

[2] S. Fukushima, K. Miyata, N. Nishiyama, N. Kanayama, Y. Yamasaki, K. Kataoka, J. Am. Chem. Soc. 127, 2810 (2005)

[3] S. Lakshmi, T.C.T Laurencin, Prog. Polym. Sci. 32, 762 (2007)

[4] K.S. Stakleff, F. Lin, L.A.S. Callahan, M.B.Wade, A. Esterle, J. Miller, M. Graham, M.L. Becker, Acta Biomaterialia 9, 5132 (2013)

[5] M.H. El-Newehy, A.S. Elsherbiny, H. Mori, J. Appl. Polym. Sci. 127, 4918 (2013)

[6] H.Wu, L. Zhu, V.P. Torchilin, Biomaterials 34, 1213 (2013)

[7] Y.A. Diaz-Fernandez, E. Mottini, L. Pasotti, E.F. Craparo, G. Cavallaro, P. Pallavicini, Biosens Bioelectron 26, 29 (2010)

[8] S. Gingter, E. Bezdushna, H. Ritter, Beilstein

J. Org. Chem. 7, 204 (2011)
[9] S. Gingter, B. Mondrzik, R. Ritter, Macromolecules 45, 1753 (2012)
[10] K. Matyjaszewschi, J. Xiao, Chem. Rev. 101, 2921 (2001)
[11] C. Boyer, V. Bulmus, T.P. Davis, V. Ladmival, J. Liu, S. Perrier, Chem. Rev. 109, 5402 (2009)
[12] S. Mallakpour, Z. Rafiee, H. Polym. Sci.: Polym. Phys. 124, 4512 (2012)
[13] I.-D. Chung, P. Britt, D. Xie, E. Harth, J. Mays, Chem. Comm. 1046 (2005)
[14] J. Skey, R. O'Reilly, J. Polym. Sci, Part A: Polym. Chem. 46, 3690 (2008)
[15] K. Bauri, S.G. Roy, S. Pant, P. De, Langmuir 29, 2764 (2013)
[16] H. Mori, H. Iwaya, A. Nagai, T. Endo, Chem. Commun. 4872 (2005)
[17] H. Mori, I. Kato, S. Saito, T. Endo, Macromolecules 43, 1289 (2010)
[18] Z. Liu, J. Hu, J. Sun, G. He, Y. Li, G. Zhang, J. Polym. Sci. Part A: Polym. Chem. 48, 3573 (2010)
[19] H. Mori, S. Okabayashi, React. Funct. Polym. 69, 441 (2009)
[20] S. Kumar, P. De, Polymer, DOI: 10.1016/j.polymer.2013.12.054
[21] H. Mori, E. Takahashi, K. Nakabayashi, Macromolecules 46, 6451 (2013)
[22] M. Yoshida, A. Safranj, H. Omichi, R. Katakai, Macromolecules 29, 2321 (1996)
[23] A. Bentolila, I. Vlodavsky, R. Isahai-Michaeli, O. Kovalchuk, C. Haloun, A.J. Domb, J. Med. Chem. 43, 2591 (2000)
[24] M. Casolaro, Macromolecules 28, 2351 (1995)
[25] S. Gingter, B. Mondrzik, H. Ritter, Macromolecules 45, 1753 (2012)
[26] W. Wu, D. Xie, A. Pucket, J.W. Mays, Eur. Polym. J. 39, 959, (2003)
[27] E.C. Buruiana, T. Buruiana, L. Hahui, J. Photochem. Photobiol. A: Chem. 189, 65 (2007)
[28] E.C. Buruiana, M. Zamfir, T. Buruiana, J. Photochem. Photobiol. A: Chem. 205, 161 (2009)
[29] R. Sjoback, J. Nygren, M. Kubista, Spectrochim. Acta Part. A 51, 7 (1995)
[30] A. Song, J. Zhang, M. Zhang, T. Shen, J. Tang, Colloid. Surface. Physicochem. Eng. Aspect. 167, 253 (1999)
[31] M. Alvarez-Pez, L. Ballesteros, E. Talavera, J. Yguerabide, J. Phys. Chem. A 105, 6320 (2001)