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Poly(ethylene glycol) functionalized by polycondensing procedure with poly(succinimide)

Summary — The paper presents the possibility to prepare the block copolymer between poly(succinimide) (PSI) and poly(ethylene glycol) (PEG) with different molecular weights of about 4000, 10 000 or 20 000. The structures of the products obtained have been confirmed by nuclear magnetic resonance (^{1}H NMR) and Fourier transform infrared spectroscopy (FT-IR) methods. The characterization of the synthesized macromolecular compounds from the viewpoint of their thermal stability, as well as the copolymers suprastructure are also presented.
Key words: poly(succinimide), poly(ethylene glycol), block copolymer, thermal stability.

GLIKOL POLIOKSYETYLENOWY FUNKCJONALIZOWANY ZA POMOCĄ POLIKONDENSACJI Z POLI(IMIDEM KWASU BURSZTYNOWEGO)

Streszczenie — Opisano syntezę kopolimerów blokowych poli(imidu kwasu bursztynowego) (PSI) z glikolem polioksyetylenowym (PEG) o różnych ciężarach cząsteczkowych (4000, 10 000 lub 20 000). Strukturę otrzymanych produktów potwierdzono metodami magnetycznego rezonansu jądrowego (^{1}H NMR, tabela 1) oraz spektroskopii w podczerwieni z transformacją Fouriera (FT-IR) (rys. 1). Określano także stabilność termiczną kopolimerów porównując ją ze stabilnością PSI (rys. 2 i 3, tabela 2). Za pomocą skaningowej mikroskopii elektronowej (SEM) badano także wpływ rodzaju użytego do syntezy PEG na nadstrukturę otrzymanych kopolimerów (rys. 4).
Słowa kluczowe: poli(imid kwasu bursztynowego), glikol polioksyetylenowy, kopolimer blokowy, stabilność termiczna.

The interest in development of biodegradable and biocompatible polymers, as for example poly(α-hydroxyl acid)s and poly(α-amino acid)s for biomedical and pharmaceutical applications has considerable increased [1]. Currently there are few synthetic and natural polymeric materials which can be used for controlled delivery of drugs, including peptide and protein drugs, because of strict regulatory compliance requirements, such as biocompatibility, clearly defined degradation pathway and safety of the degradation products [2—4]. Among these, the most commonly used hydrophilic blocks comprise of poly(ethylene glycol) (PEG) with the monomer subunit $-CH_2-CH_2-O-$, the end groups depending on the synthesis procedure most often being hydroxyl or methoxy groups. PEG is used due to its biocompatibility, minimal toxicity and antigenicity, and good solubility in water or common solvents. Surfaces able to resist the protein adhesion and biological attack were obtained using PEG as a surface protector. Grafting PEG to solid surfaces reduces protein adsorption and cell adhesion [5—9]. PEG coatings suppress also platelet adhesion and lead to reduced risk of thrombus formation, tissue damage, and other cytotoxic effects [10]. At the same time the polymers for bioapplications may have pendant functional groups to which drugs or biologically active compounds could be covalently attached. PEG does not meet this requirement. Synthetic polymers containing amino acid residues in the main chain or in the side chain are some examples of functional polymers for biomedical use.

Poly(aspartic acid) (PAS), belonging to the family of synthetic polypeptides, is a biocompatible and biodegradable water soluble polymer. Partly due to the carboxylic groups, PAS has some similarity in chemical properties with poly(acrylic acid) [11]. As biocompatible compound, with no toxic or mutagenic effect, PAS can be used in medicine, cosmetics and food industry. It is also considered as a sustainable and environmentally friendly chemical product due to its biodegradability that makes it particularly valuable from the viewpoint of the environmental acceptability and waste disposal [12—15].

Particularly, poly(succinimide) (PSI), as PAS precursor, is one of the most promising particle carriers since it was demonstrated to have suitable physicochemical characteristics for development of macromolecular prodrugs, due to biocompatibility, biodegradability, and ease of synthesis and functionalization [5—11].

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A supramolecular structure formed through macromolecular assembly from PAS and PEG has recently received considerable attention in various aspects in both fundamental and applied fields of polymer science. This kind of polyion complex (PIC) micelle formed by a block copolymer with core-shell morphology is one on which intensive studies have been carried out especially due to their use in many applications, including drug delivery systems, separation technologies, and optoelectronic devices [16].

In our previous papers it was presented preparation of an interpolymer complex (IPC) obtained by complexation *via* hydrogen bonding between PAS and poly(vinyl alcohol) [17—20]. The IPC was subsequently used for the bioactive substances entrapment, respectively it was doped with silver nanoparticles. The miscibility and compatibility between the polymeric components was confirmed by dynamic rheology and zeta potential analyses. At the same time, the influence of the polymer structure and the ratio between the components brought additional possibility of the hydrogen-bonded complexation. The homogeneity of IPC based on PAS and PEG investigated with near-infrared chemical imaging (NIR-CI) technique was also presented [21]. The data were obtained by assessment of compatibility between the polymeric compounds through dynamic rheology and zeta potential analyses.

In the present paper the possibility to prepare the block copolymer between PSI and PEG (with different molecular weights of about 4000, 10 000 or 20 000) is presented. There are presented characterization of the synthesized macromolecular compound from the point of view of their thermal stability, as well as the copolymers suprastructure by scanning electron microscopy (SEM).

EXPERIMENTAL

Materials

Dodecane (Fluka Chemika, purity >90 %), DMF (Fluka Analytical, purity >98 %), L-aspartic acid (Fluka BioChemika), *o*-phosphoric acid (analytical reagent, 85 % purity, Chemical Co. Romania), manganese(II) acetate (Sigma Aldrich, purity 98 %) were used as received. Three samples of poly(ethylene glycol (PEG) (purchased from Fluka, Germany) without further purification, with the molecular weight of about 4000, 10 000, and 20 000 (PEG_{4000}, $PEG_{10\,000}$ and $PEG_{20\,000}$, respectively) were applied in the reaction.

Synthesis procedure

In the first step, PSI was synthesized by thermal polycondensation of L-aspartic acid in dodecane, at 180 °C for 6 h with *o*-phosphoric acid as catalyst. In an usual experiment, the reaction was performed in a 250 cm^3 three necked round-bottom flask equipped with mechanical stirrer, a Dean-Stark trap fitted on water condenser, with thermometer in the vapor circuit, and heated in a thermoregulated oil bath.

In the second step the copolymer PSI-*co*-PEG was synthesized. The same equipment was charged with 30 cm^3 of dimethylformamide (DMF) as reaction medium, 4 g of PSI and 2 g of PEG (with certain molecular weight), grounded to fine powder form in a porcelain mortar.

The mixture was heated at 138 °C and 0.09 g of manganese(II) acetate as catalyst dissolved in 5 cm^3 of water was added from a weeping funnel for 1 h. At this temperature, the reaction mixture was maintained for another 6 h under vigorous continuous stirring. During this time 8 cm^3 of formed water was collected, what confirmed that polycondensation process occurred [see eq. (1)].

Finally, the reaction flask was cooled down to the room temperature. The polymer was separated by sedimentation, washed three times with methanol. Then it was filtered and dried in a vacuum oven at 50 °C.

Analysis methods

Nuclear magnetic resonance (^{1}H NMR, 300 MHz) spectra were recorded from the studied samples dissolved in dimethyl sulfoxide (DMSO) on a Bruker AM-300 spectrometer.

The molecular structures was investigated using Fourier transform infrared spectroscopy (FT-IR). DIGILAB spectrophotometer (Scimitar Series, USA), with recording resolution 4 cm^{-1} has been used (concentration was 3 mg sample in 500 mg KCl tablet).

Thermogravimetric analysis (TGA) was performed under nitrogen atmosphere in the temperature range between 30 and 700 °C at heating rate 20 deg/min with a TGA/SDTA 851 Mettler Toledo instrument. Two experiments were made for each formulation.

Scanning electron microscopy (SEM) studies were performed on samples fixed by means of colloidal copper supports. The samples were covered by sputtering with a thin layer of gold (EMITECH K 550x). The coated surface was examined using an environmental scanning electron microscope (ESEM) type Quanta 200 operating at 30 kV with secondary electrons in high vacuum mode.

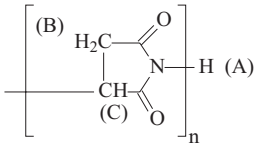
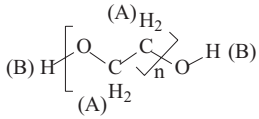
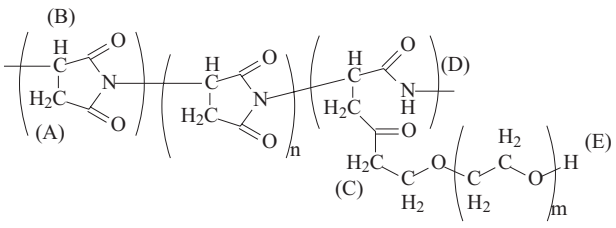
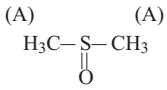
RESULTS AND DISCUSSION

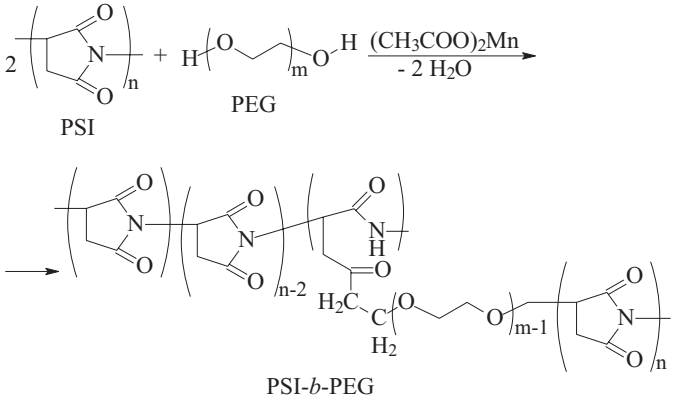
PSI-*b*-PEG copolymers were synthesized according to the Scheme A.

PSI was directly reacted with PEG to prepare the block copolymers of PSI-*b*-PEG. PSI and PEG_{4000}, as well as prepared copolymer PSI-*b*-PEG_{4000} were investigated using ^{1}H NMR method. The registered signals and their assignments are presented in Table 1 and they are in good conformity with the literature data [22].

The spectra confirm formation of the PSI-*b*-PEG block copolymer. Thus the signals of protons at 2.5, 2.7, 2.8, 3.25, 3.1—3.25 ppm are attributed to the methylene car-

T a b l e 1. ^{1}H NMR chemical shifts of PSI, PEG_{4000} and prepared PSI-*b*-PEG_{4000} copolymer

Compound	Assignment	Shift (ppm)
 PSI	A	8.9
	B	2.8, 3.2
	C	5.3
 PEG_{4000}	A	3.4, 3.5
	B	2.5
 PSI-*b*-PEG_{4000}	A	2.7, 2.8, 3.25, 3.1—3.25
	B	3.6—3.8, 5.25—5.5
	C	2.5
	D	7.9, 8.1, 8.3, 8.7
	E	2.2
 DMSO	A	1.25



Scheme A. The idealized synthetic route for poly(succinimide)-b-poly(ethylene glycol)

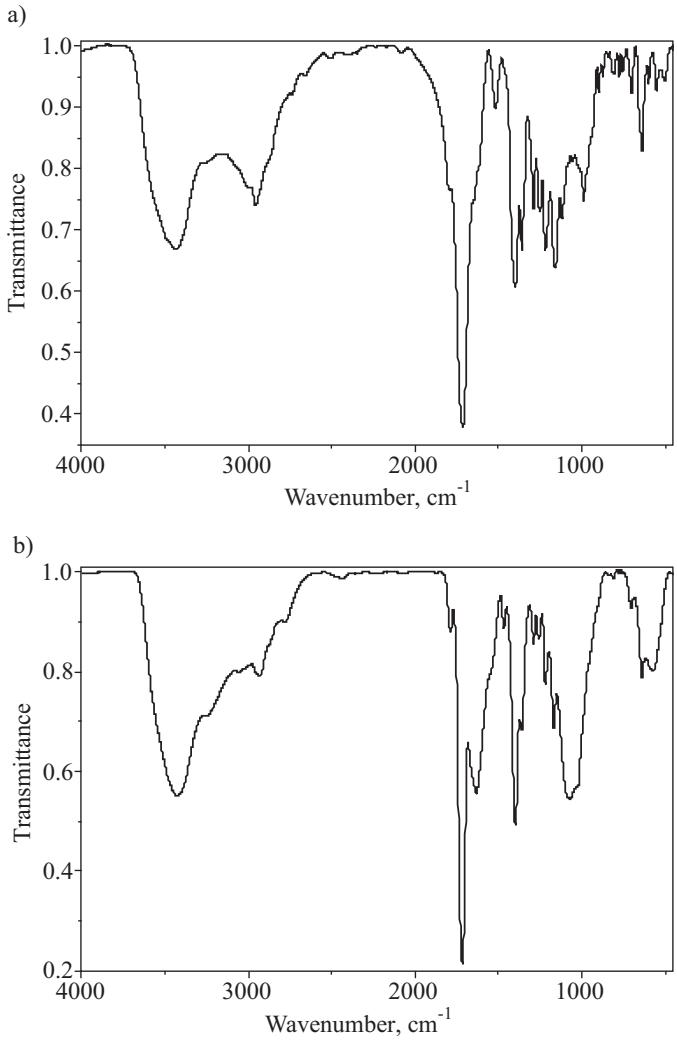


Fig. 1. FT-IR spectra of: a) PSI, b) PSI-b-PEG_{4000}

bon from succinimide and ethylene glycol units, respectively. The methine protons signals from 3.6—3.8 and 5.25—5.5 are thought to be coupled to the amide protons ascribed to the 7.9, 8.1, 8.3, 8.7 ppm region.

The FT-IR spectra of PSI and prepared PSI-*b*-PEG_{4000}, illustrated in Fig. 1, sustain the ^{1}H NMR data confirming formation of the block copolymer.

Thus FT-IR spectrum of PSI (Fig. 1a) include the CH stretch at about 3000 cm^{-1}, the carbonyl νC=O from cyclic imides stretch in the region of 1700 cm^{-1} [23], around 1270 cm^{-1} are registered vibrations corresponding to νCN amide band III and the CH_2 band shows up at approximately 1400 cm^{-1}. A very broad peak in the region between 3100 and 3600 cm^{-1} indicates presence of amide groups.

The spectrum of PSI-*b*-PEG_{4000} (Fig. 1b) is characterized by presence of the bands typical for both compo-

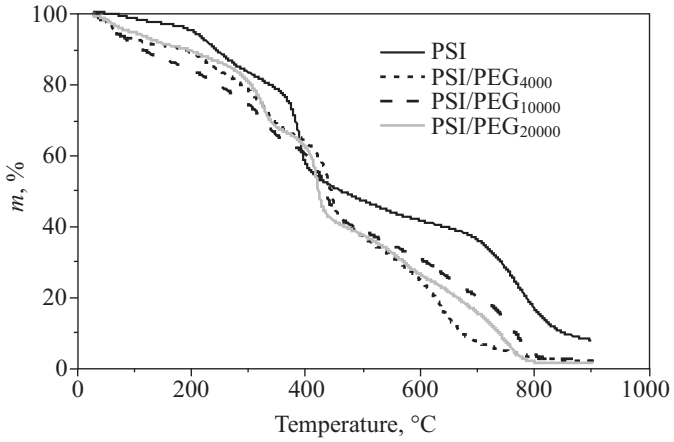


Fig. 2. TG curves of PSI and of the prepared block copolymers

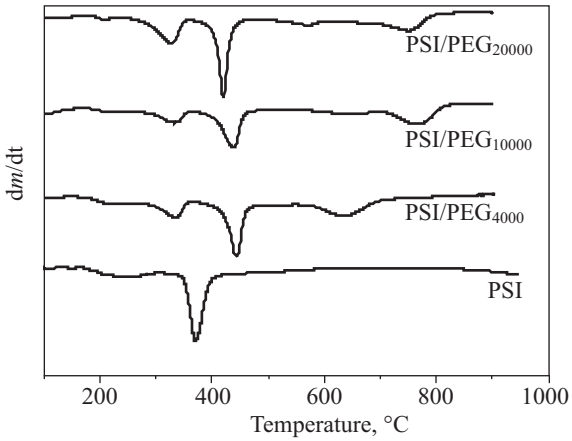


Fig. 3. DTG curves of PSI and of the prepared block copolymers

nents confirming the block copolymerization process. Consequently, from this spectrum must be mentioned: the band around of 3500 cm^{-1} which is attributed to O-H stretch as well as to the amide stretch, found again around 1660 cm^{-1}, the CH stretch bands near 3000 cm^{-1}, the CH_2 band enlarged in the region of 1400 cm^{-1} and again the band of carbonyl CO stretch near 1700 cm^{-1}.

There are no registered differences between spectra owing to the differences of the PEG molecular weights used for preparation of the block copolymer. Some shifting of the characteristic bands is visible between the spectra because of other occurring phenomena as for example implication of the functional groups into H-bonds.

The results of the preliminary characterization of the prepared block copolymer and its constitutive components — PSI and PEG — are completed with some aspect concerning their behavior during thermal analyses. As it can be seen from Figures 2 and 3 and results listed in Table 2 there were also found differences between the thermal behavior of the prepared copolymer and PSI.

PSI presents a thermal decomposition process in three stages and lower thermal stability than PSI-*b*-PEG. The superior thermal stability is attributed to physical links intervened between the macromolecular chains, as for example hydrogen bonds. Generally, mass loss recorded in the first thermal decomposition process (up to 300 °C) is approximately 4 % and it is attributed to elimination of adsorbed water and to the dehydration processes [24]. The literature data mention that PEG loses mass in one step during its thermal degradation and more species having low molecular weight were obtained [25]. The mechanism of this process follows the conventional random chain scission.

In the case of prepared block copolymers the behavior during thermal analysis is more complex. The thermal decomposition processes demonstrates significant mass losses in every interval of temperature. Thus in the first part of the degradation process (until 260 °C), water and some easily volatile products are eliminated (approximately 12 %) and in 260—580 °C temperature interval there exist other thermal decomposition processes, with mass losses caused by C-C linkages scission.

The prepared block copolymers were also analyzed for their supramolecular structure.

T a b l e 2. Characteristic temperatures corresponding to thermal decomposition steps

Sample	First step				Second main step				Third step				T_{10} °C	T_{50} °C	$\Sigma\Delta m$ %
	T_i*) °C	T_{max} °C	T_f °C	Δm %	T_i °C	T_{max} °C	T_f °C	Δm %	T_i °C	T_{max} °C	T_f °C	Δm %			
PSI/PEG_{4000}	305	334	406	32	406	443	461	26	461	633	702	37	167	421	95
PSI/$PEG_{10\,000}$	318	336	405	35	405	438	458	22	458	770	820	40	110	436	97
PSI/$PEG_{20\,000}$	305	326	395	33	395	420	444	26	444	760	833	38	176	420	97
PSI	200	234	300	16	300	390	450	34	450	774	892	41	247	467	91

*) T_i, T_f — onset and final temperature of the thermal decomposition step, T_{max} — maximum temperature for the mass losses at given step, Δm — mass losses determined on T_i—T_{max} interval, T_{10}, T_{50} — the temperatures corresponding to 20 and 50 % mass losses, respectively, $\Sigma\Delta m$ — mass losses during all three steps of decomposition.

Figure 4 presents the differences registered in morphological structures of the polymeric matrices studied which are demonstrated with SEM technique. There were shown morphological changes between the samples. Thus the structure is like a shell coating in the case of PSI-*b*-$PEG_{20\,000}$ and as a "fish skin" in the case of PSI-*b*-PEG_{4000}. This aspect is attributed exclusively to different molecular weight of PEG which becomes capable to form a film along with growth of its molecular weight.

The advantages of these structures are their biodegradability as well as their biocompatibility. Due to

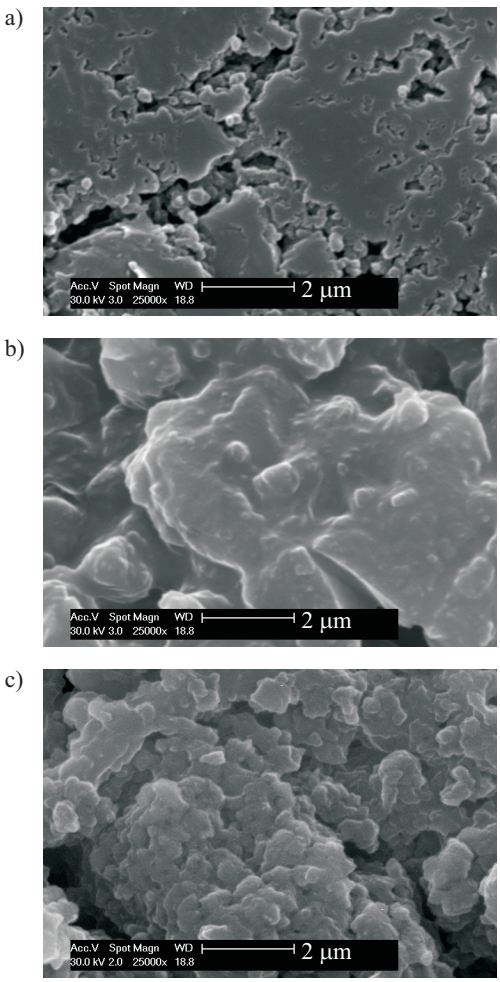


Fig. 4. SEM micrographs of the block copolymers: a) PSI-b-PEG$_{20\,000}$, b) PSI-b-PEG$_{10\,000}$, c) PSI-b-PEG$_{4000}$

these characteristic they can be used as matrix for bioactive products.

CONCLUSIONS

The structure of the prepared PSI-*b*-PEG block copolymers was confirmed by ^{1}H NMR spectra as well as FT-IR spectra. The study confirmed higher thermal stability of the PSI-*b*-PEG copolymers in comparison to PSI homopolymer attributed to physical links intervened between the macromolecular copolymer chains, as for example hydrogen bonds. There are also shown morphological differences among the block copolymers due to different molecular weights of PEG included into the block. Thus the structure is like a shell coating in the case of PSI-*b*-PEG$_{20\,000}$ and as a "fish skin" in the case of PSI-*b*-PEG$_{4000}$.

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