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Characterization and Properties of Polyesteramides Containing Long-chain n-alkyl Substituents

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Abstract

On the basis of natural aminoacids: L-leucine, L-phenylalanine, L-lysine are received co-polyesteramides containing n-alkyl groups (octyl (Oct), dodecyl (Dod) and hexadecyl (Hex)) in AABB-type lateral chain. Co- polyesteramides are received virtually in quanti-ta-tive yield, with very high molecular masses and narrow molecular-mass distribution. This is an important fact from the viewpoint of their use for the biomedical purposes. Mechan-ical properties of some polymers are studied by us. Study of thermal and me-chanical properties of polymers is important for polymers for biomedicine purposes, since they have impact on many other properties of polymers, including biodegradation capaci-ty and they are respectively precondition for the sphere of their application.

Keywords: Co-Polyesteramides, Octyl (Oct), Dodecyl (Dod), Hexadecyl (Hex), N-Alkyl Group, Domain

Introduction

Among polymeric materials for the biomedicine purposes, which are successfully used in many fields of medicine, are especially noteworthy the materials for systems of purposeful, uninterrupted/ controllable delivery of physiologically active materials, since these systems give an opportunity of solving the most difficult problems of medicine (Liechty, Kryscio, Slaughter, Peppas, 2010; Peppas, Hoffman, Kanamori, Tojo, 2006).

As it is known, the use of many preparations is limited due to their low stability, toxicity or non-selective action. Some prepara-tions are removed very fast from the body hereupon it is be-coming necessary to increase their dosage or frequency of admin-istration (dosing). Solution of the mentioned problems is possible through a delivery of medicinal preparation with the use of high-molecu-lar carriers. Polymeric carriers' function is not limited only by drug transportation. Frequently they protect drugs and other biolog-ically active materials (e.g. ferments) from inactivation, implement func-tion of reservoir from where gradual release of drug occurs, in-creases their selectivity, and etc. (Liechty, Kryscio, Slaughter, Pep-pas, 2010; Peppas, Hoffman, Kanamori, Tojo, 2006).

Non-covalent binding is an interesting method of preparation delivery. In this case interaction between molecules is implement-ed by non-covalent bonds among which hydrophobic interaction is one of the most prospective ones. Despite the fact that hydrophil-ic-hy-drophobic interaction regulates a lot of biological processes in syn-thetic chemistry, amphiphilic compounds are relatively less studied. Only recently were focused an attention on the signifi-cance of hy-drophobic interaction, especially for receipt of medic-inal prepara-tions' purposeful delivery systems (Katsarava, Kha-radze, Jokha-dze, Neparidze, 2009). Both, natural (reconstructed) and synthetic liposomes, micelles, structure of core-shell type and block-copoly-mers etc. are spectacular examples of the use of hy-drophilic-hydro-phobic interactions (Katsarava, Kharadze, Jokha-dze, Neparidze, 2009; Sahoo, Dilnawaz, Krishnakumar, 2008). By using them it is possible to transport medicinal preparations to cell membrane and hematoencephalic barrier.

On the basis of polymeric natural aminoacids of biodegrad-able AABB type with high bio-compatibility skills, synthesis and system-atic study of which are carried out in Georgia (Katsarava, 1989; Kharadze, 1998), are received prospective compounds in the form of matrixes of medicinal preparations. Bio-compatibili-ty capacity and low immunogenic properties of these polymers are conditioned by monomers' nature and structure of polymeric chains. Among polymers of different classes, received on the basis of natural ami-noacids are especially noteworthy polyesteramides of AABB type, on the ground of which are obtained a lot of poly-

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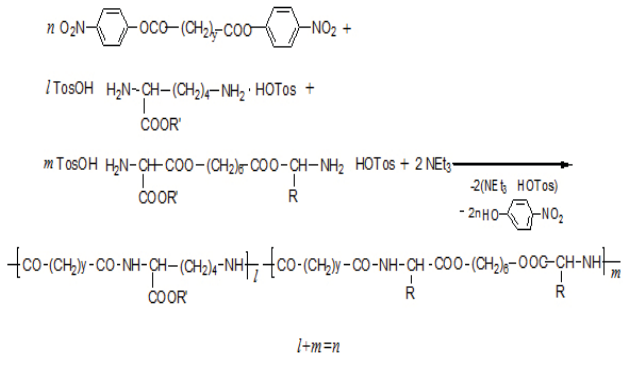
meric preparations with medicinal properties. For instance, one of these preparations is biodegradable polyesteramide, alongside of which the iminoxyl radical is fixed by covalent bond (Lee, Szinai, Carpenter, Katsar-ava, Jokhadze, Chu, Huang, Verbeken, Bramwell, DeScheerder, Hong, 2002). Production of the mentioned preparations received in Georgia was carried out by US biotechnological company MediVas in the form of blood-vascular stent covering material (for suppres-sion of restenosis) working according to the controllable separation mechanism. In general, regulation of biodegradation capabilities and physical-mechanical properties of polyesteramides obtained on the basis of aminoacids is possible in wide range thanks to di-versity of aminoacids and their derivatives and due to synthesis capability of polymers and copolymers of various classes on their basis. For identifying the sphere of application of polymers for bio-medical purpose along with other features are important synthe-sis and research of polymers, as well as the study of their thermal properties, since phase state of polymers has important effect on other properties of polymers, e.g. on biodegradation capabilities and, respectively, it changes the area of their use.

For instance, biodegradation of crystal polymers proceeds at far less rate than amorphous polymers. Polymers with high vitrifica-tion temperature are prospective compounds as surgical and struc-tural materials, while polymers with low vitrification temperature are useful as artificial leather, stent covering materials for receipt of medication controllable separation systems.

Methodology

On the basis of natural aminoacids: L-leucine, L-phenylalanine, L-lysine are received co- polyesteramides containing n-alkyl groups (octyl (Oct), dodecyl (Dod) and hexadecyl (Hex)) in AABB-type lateral chain. Synthesis of co- polyesteramides [coPEA(Alk)] con-taining lateral alkyl long-chain substitutes was conducted through polycondensation of di-p-toluene-sulphoacid salts of bis-α-amino-acid-α,ω-alkylene-diesters, and di-p-toluene-sulphoacid salts of L-lysine long-chain alkyl esters as co-polymers with p-nitrophenyl adipate or p-nitrophenyl sebacate in the aprotonic amid-type solvent, under optimal conditions established for homo-polyamides.



where X=6; Y=4;8

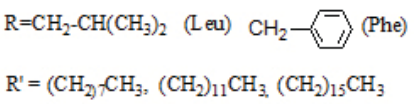


Figure 1. Synthesis of co- polyesteramides [coPEA(Alk)] containing lateral alkyl long-chain substitutes

Di-p-toluene-sulphoacid salts of bis-α-aminoacid-α,ω-alkylene-diesters and L-lysine long-chain alkyl esters are received by us in different ratios (l/m), according to which we have regulated the amount of lateral 'adhesive' hydrophobic groups, on one side, and concentration etheric bonds in basic chain of macromolecule and thereby physical-chemical properties of obtained polymers. Separation of polymers from the reaction site was made by precipitation in water medium. Precipitated polymers were washed during several days in order to remove solvent (DMA) and low-molecular products of polycondensation (n-nitrophenol and NEt3·HOTos), as a result of which it was gradually transformed into resinous solid or elastic rubber-like mass (depending on structure).

Polymers were dried in vacuum at 20-25°C and for final purifi-cation were precipitated from chloroform solution in ethylacetate. It should be noted that co-product of polycondensation – p-nitro-phenyl is difficult to remove from polymer. Polymer purity mon-itoring was conducted by us by means of UV-spectrum, till the disappearance of p-nitrophen-oxide anion's absorption band in 430 nm region.

For controllable/uninterrupted drug separation system it is im-portant for the polymer carriers to be biodegradable and at the same time quite hydrophilic in order to prevent fast washout of drugs. Co- polyesteramides were selected by us for the accom-plishment of this goal, since their properties can be regulated by the variation of molar ratio of dicarboxylic acids, diols and hydro-phobic aminoacids (from which biodegradable part of macrochain is built), on the one hand, and lysine alkyl esters (which are used for insertion of hydrophobic 'adhesive' groups), on the other. α,ω-al-kylene-diester received on the basis of leucine adds elasticity to macrochains, while α,ω-alkylene-diester of phenylalanine gives them rigidity. Insertion of lysine fragments to the basic chain of co- polyesteramides causes increase in molar concentration of amide bonds. All the above mentioned was taken into account during the synthesis of co- polyesteramides and the selection of molar ratio of monomers and co-monomers.

Results

Our basic goal was a study of effect of n-alkyl, in particular octyl, dodecyl and hexadecyl groups on physical and mechanical proper-ties of co- polyesteramides.

Characteristics of obtained co- polyesteramides are given in Table 1. Probable structure of co- polyesteramides received on the basis of lysine long-chain alkyl esters is confirmed by elemental analysis data (coincidence between calculated and found per-centage contents of elements is virtually ideal). Probable structure was also established by us with the use of Furrier IR-spectroscopy (Figure 2.), and by way of illustration a sample of polyesteramide 8-[Leu-6]0.80[lys(Dod)]0.20 is given in the text. Absorption bands of amide NH (3300 cm-1), etheric CO (1730-1740 cm-1), and amide CO (1660 cm-1) are seen in the spectrum, at the same time, an intensity of etheric carbonyl and amide bonds, in contrast with polyamides, is almost equal that is explained by increase in con-centration of etheric bonds in co- polyesteramide chain. Spectra of polyamides and polyesteramides are very similar to each other (as was expected), and only insufficient difference is observed in fin-gerprint area. As it is clearly seen from the Table, with the use of the above mentioned method polymers are obtained by virtually quan-titative yields. All obtained co- polyesteramides are distinguished by film-formation skill. Elastic films were received by us through pouring of polymer solution (in chloroform) on hydrophobic surface. It should be noted that leucine-containing co- polyesteramides are not characterized by adhesiveness and, correspondingly, films do not become adhesive while storing.

As it can be seen from Table 1, co- polyesteramides are received virtually in quantitative yield, with very high molecular masses and narrow molecular-mass distribution that is important fact from the viewpoint of their use for biomedical purposes.

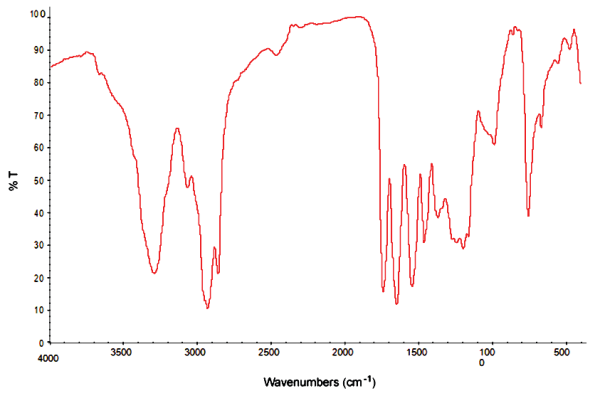


Figure 2. Infrared spectrum of co- polyesteramide 8-[Leu-6]0.80[lys(Dod)]0.20

(on KBr plate)

Table 1. Characteristics of co- polyesteramides received on the basis of lysine long-chain alkyl ethers.

#	Co-polyesteramides	Yield %	η_{red}, dL/g	M_w	M_w/M_n	Elemental analysis, calculated ,% found, %		
						C	H	N
1	4-[Leu-6]$_{0.8}$[Lys(Oct)]$_{0.2}$	98	0,49	65000	1,25	63.22 63,71	9.15 9,4	6.67 6,4
2	4-[Leu-6]$_{0.6}$[Lys(Oct)$_{0.4}$	97	0,46	65800	1,25	64 44 64,03	9.11 9,50	6.40 6,67
3	4-[Leu-6]$_{0.4}$[Lys(Oct)]$_{0.6}$	99	0,44	57100	1,27	64.76 64,38	9.43 9,61	6.84 6,95
4	8-[Leu-6]$_{0.8}$[Lys-Oct)]$_{0.2}$	96	0,46	66200	1,28	65,98 66,20	9,46 9,97	5,76 5,68
5	8-[Leu-6]$_{0.8}$ [Lys(Dod)]$_{0.2}$	99	0,40	44800	1,29	66,38 66,63	9,87 10,07	5,58 5,55
6	8-[Leu-6]$_{0.8}$[Lys(Hex)] $_{0.2}$	100	0,41	45000	1,26	67.34 67,05	9.98 10,16	5.65 5,43
7	4-[Phe-6]$_{0.8}$[Lys(Oct)]$_{0.2}$	98	0,11	25000	1,25	68.70 68,38	7.55 7,71	5,87 5,70
8	4-[Phe-6]$_{0.4}$[Lys(Oct)$_{0.6}$	98	-	-	-	66. 78 67,01	8.47 8,62	6.44 6,51
9	4-[Phe-6]$_{0.2}$[Lys(Oct)]$_{0.8}$	99	-	-	-	66. 56 66,17	8.99 9,19	6.89 7,02
10	8-[Phe-6]$_{0.8}$[Lys(Oct)]$_{0.2}$	97	0,37	41600	1,27	70.57 70,15	8.45 8,39	5.02 5,11
11	8-[Phe-6]$_{0.4}$[Lys(Oct)$_{0.6}$	98	0,47	70000	1,5	68.87 69,16	9.38 9,29	5.55 5,76
12	8-[Phe-6]$_{0.8}$[Lys(Dod)]$_{0.2}$	99	0,29	32400	1,17	70.23 70,46	8.45 8,51	5.16 5,01
13	8-[Phe-6]$_{0.6}$[Lys(Dod)]$_{0.4}$	100	0,19	22700	1,13	70.11 70,35	8.97 9,04	4.89 5,19
14	8-[Phe-6]$_{0.4}$[Lys(Dod)]$_{0.6}$	99	*	*	*	70.56 70,23	9.58 9.62	5.12 5.39
15	8-[Phe-6]$_{0.8}$[Lys(Hex)]$_{0.2}$	99	0,35	32900	1,38	70,56 70,76	8.43 8,62	4,78 4,91
16	8-[Phe-6]$_{0.8}$[Lys(Hex)]$_{0.4}$	100	*	*	*	70,91 70,96	9,52 9,25	4,86 4,98
17	8-[Phe-6]$_{0.4}$[Lys(Hex)]$_{0.6}$	100	*	*	*	71,45 71,16	9,88 9,90	5,34 5,06

*) is not dissolved in DMF at room temperature;
-) is not measured

Obtained co- polyesteramides were characterized by reduced viscosity, identified nuclear-magnetic resonance spectra (1H). Latter method is the most informative and frequently makes possible microstructural analysis of polymers. By way of illustration, NMR-spectra of two samples (Fig.4., Fig.5.,Table 2., Table 3.) are given by us in the text, e.g. for polymers:

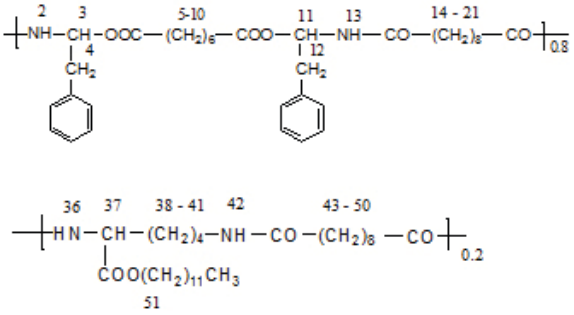


Figure 3. Synthesis of co- polyesteramide 8-[Phe-6]0.8[Lys(Dod)]0.2

In 1H spectra of these polymers is clearly seen the complex multiplet of monosubstituted benzene nucleus, with chemical shift 7,05-7,25 ppm; triplet signal of hydrogen located close to 3rd carbon atom with chemical shift 4,55 ppm (J1 =6,9 Hz) and triplet of methine hydrogen of lysine CH-CH2 fragment, with chemical shift 7,05-7,25 ppm 4,51 ppm (J=7,9 Hz), methylene hydrogen of phe-nylalanine, with chemical shift 3,05 (J-4,8 Hz), 2,98 (J=7,14 Hz) and methylene hydrogen of lysine, with chemical shift 3,91 ppm (J=9,5 Hz). Triplet signal with chemical shift 4,06 ppm (J=7,1 Hz) belongs to methylene hydrogens of ester group. Methyl group resonates in the strongest part of field 0,86 ppm (J=9Hz). Chemical shift of 41st proton equals to 2,9 ppm (J=9,52 and 3,3 Hz). Belonging of signals located in the strong field is given below.

Table 2. Chemical shifts (ppm) and constants of spin-spin interaction J (Hz)

NH (2,13)	H-3 H-11	H-4	H-5 H-10	H-12	Ph	NH-36	NH-42	H-14;21 H-43;50	H-41
7,9 Doublet J = 6,5 Hz	4,55 Doublet-triplet J = 6,9 Hz	3,08 Doublet J = 5,5 Hz 2,98 Doublet J = 6,9 Hz	2,05 Triplet J = 6,9 Hz	2,92 Doublet J = 9,52 Hz 2,88 Doublet J = 9,52 Hz	7,05-7,25 Complex multiplet	7,76 Doublet J = 4,76 Hz	7,42 Triplet J = 2,9 Hz	2,15 Triplet J = 7,1 Hz	2,9 Triplet J = 6,9 Hz

of polyesteramide 8-[Phe-6]0.8[Lys(Dod)]0.2.

1 Constant of spin-spin interaction

NH19	H-29	H-15	H-16	H-17	H-18	H-13 H- 43	NH-28	H-35 H-42	NH-34	H-33
7,8 Doublet J = 8,0 Hz	4,15 Doublet-triplet J = 6,71 Hz J = 5,58 Hz	2,1 Triplet J = 8,4 Hz	2,0-2,2 Complex multiplet	0,84 Doublet J = 7,5 Hz	0,92 Doublet J = 7,5 Hz	4,02 Triplet J = 6,9 Hz	7,55 Extended singlet	2,85 Extended singlet	7,42 Triplet J = 5,85 Hz	3,06 Triplet J = 7,5 Hz

Table 3. Chemical shifts (ppm) and constants of spin-spin interaction J (Hz) of polyesteramide 8-[Leu-6]0.8[Lys(Oct)]0.2

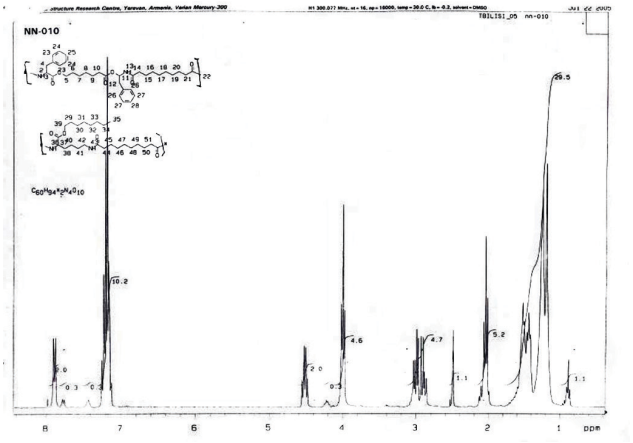


Figure 4. 1H NMR spectrum of polyesteramide 8-[Phe-6]0.8[Lys(Dod)]0.2

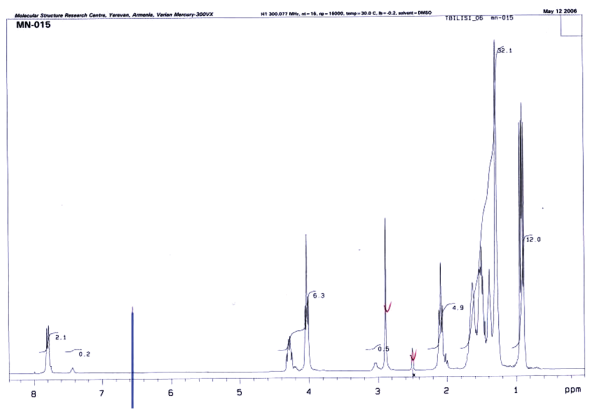


Figure 5. 1H NMR spectrum of polyesteramide 8-[Leu-6]0.8[Lys(Oct)]0.2

As to amide hydrogen, its value in phenylalanine fragment is equal to 7,9 ppm, while in case of lysine - 7,8 ppm and 7,4 ppm. Resonance signals with chemical shift 7,9 and 7,8ppm belong to different elemental ring and can be used for determination of ele-mental ring ratio. Ratio of integral intensities of mentioned signals is 20:5=4:1 that is in good agreement with represented structure of polymer (theoretical ratio = 4:1).

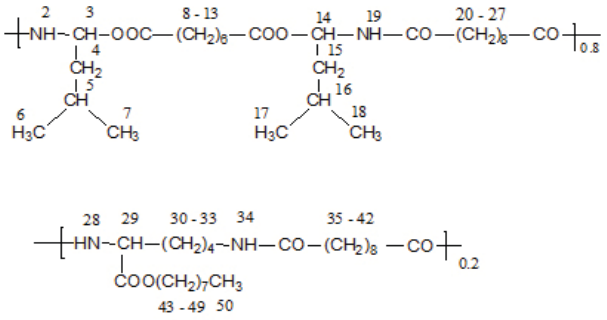


Figure 6. Synthesis of co- polyesteramide 8-[Leu-6]0.8[Lys(Oct)]0.2

In spectrum (Fig. 5) leucine residue is manifested in the form of two strong-field doublets with chemical shift 0,92 and 0,84 ppm, J = 7,5 Hz. Respective methine proton in the form of complex multiplet resonates at 2,0-2,2 ppm. Methylene protons of leucine resonate in the same region in the form of two overlapped doublets, with center at 2,1 ppm and J = 8,4 Hz. As to methine proton bonded with aminogroup, its chemical shift equals to 4,15 ppm, while J=6,5 Hz. From the rest of signals must be selected triplet signal of CH2 group (with chemical shift 4,02 ppm, J=7,5 Hz) located close to ester group. Belonging of respective signals of the rest of protons is given in Table 3.

For determination of ratio of repeatable rings, the ratio of in-tensity of aminoacid aminogroups have been used. In our case, it equals to 122:30=4:1 which is in good agreement with theoretical ratio.

Polyesteramides synthesized by us (Neparidze, Chkhaidze, Tabidze, Siradze, Kharadze, Katsarava, 2015) contain long lateral alkyl group and may be considered as comb-like polymers.

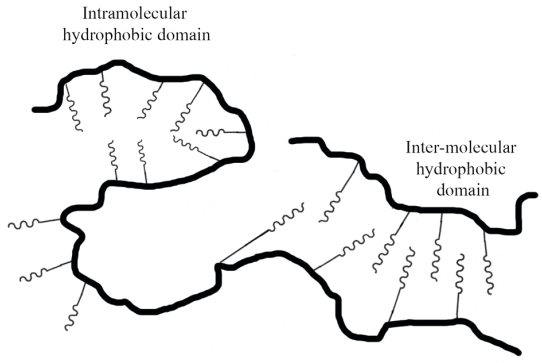


Figure 7. Structure of synthesized polyesteramides

It was expected that these lateral groups precondition strong hydrophobic interaction (both intra- and intermolecular interactions, see diagram given above) that in its turn was a reason of supra-mo-lecular (super molecular) structure of polymers and, respectively, had an effect on thermal properties of polymers. That is why we decided to conduct systematic colorimetric study of synthesized co- polyesteramides.

Polymers' properties can be assessed by change of vitrification temperature (Tg) and specific heat capacity (Δcp). Tg increases in case of limitation of intra- and extramolecular flexibility of polymeric chains, while Δcp shows us what will follow conformation change of macromolecules – reduction (Δcp increases) or increase (Δcp reduces) of free energy of amorphous part. Thermal studies also show whether any formation of crystal phase or other kinds of highly organized domains take place. Also we can note that the study of thermal and mechanical properties of polymers is im-port-ant for polymers for biomedicine purposes, since they have impact on many other properties of polymers, including biodegradation capacity and they respectively precondition the sphere of their ap-plication.

Mechanical properties of some polymers are studied by us.

Polymer	σ, MPa	ε, %	E, GPa
8-[Phe-6]$_{0,9}$[Leu-6]$_{0,1}$	14,4	315	0,4
8-[Phe-6]$_{0,8}$[Leu-6]$_{0,2}$	8,2	30	1,58
4-[Phe-6]$_{0,8}$[Leu-6]$_{0,2}$	20,7	300	1,2
4-[Phe-6]$_{0,4}$[Leu-6]$_{0,6}$	Fragile		

Table 4. Mechanical properties of polyesteramides

σ, MPa – abruption limit; ε, % - relative elongation; E, GPa – Young's modulus.

Conclusion

As obtained data show, high-elasticity properties are charac-teristic for polyesteramides 8-[Phe-6]0,9[Leu-6]0,1 and 4-[Phe-some their properties gave us an opportunity to make conclusion that obtained polymers due to content of lateral n-alkyl substitutes (presence of well-ordered domains) make the above mentioned polymers prospective for constructing the systems of purposeful uninterrupted/ controllable delivery of medicinal preparations and physiologically active materials.

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