NMR Investigation of Biodegradable Polyesters for Medical Applications

Janusz Kasperczyk

Centre of Polymer Chemistry PASc, ul.M.C.Sklodowskiej 34, 41-800 Zabrze, Poland

SUMMARY: Results of NMR studies of chain microstructure in polylactide, poly(lactide-co-ε-caprolactone), poly(lactide-co-glycolide) and poly(glycolide-ε-caprolactone) are presented. The appropriate conditions of polymerization: temperature, solvents, type of initiators allows moulding of the structure of obtained polyesters. Statistical analysis of growing chains helps to identify in NMR spectra signals of the random and block segments of copolyester chains. Precise control of reaction conditions allows modification of chain structures by affecting rates of transesterification. Equations allowing for quantitative determination of the role of transesterification reactions on the basis of intensities of NMR resonance lines are given. Changes in the microstructure of macromolecular chains were determined using calculated values of transesterification coefficients, degree of randomness and values of average lengths of syndiotactic or isotactic blocks in lactide homopolymers or average lengths of comonomer units in copolymers.

Introduction

In last years studies of medical (*in vivo*) applications of biodegradable polymeric materials constitute a continuously growing field of research. Such materials have to satisfy all medical requirements: good mechanical and physical properties, compatibility with tissue and blood, possibility of degradation to non-toxic products [1,2]. The polymers, which are used as degradable materials, should have hydrolytically unstable chemical bonds in the main chain. Polyesters obtained from glycolide, lactide and ε-caprolactone satisfy the above requirements. Various applications require production of polyesters with new physical and mechanical properties; in case of drug releasing systems rigorous monitoring of polymeric material degradation and drug release time are required. Physical properties of polyesters obtained from the mentioned above monomers are strongly connected with microstructure of their chains.

 CCC 1022-1360/00/$ 17.50+.50/0

With purpose to simplify notation of chain structures the following symbols will be used in this paper:

glycolyl unit	**G**	**-(O-CH_2-CO-)-**
glycolidyl unit	**GG**	**-(O-CH_2-CO-O-CH_2-CO-)-**
lactyl unit	**L**	**-(O-$CHCH_3$-CO-)-**
lactidyl unit	**LL**	**-(O-$CHCH_3$-CO-O-$CHCH_3$-CO-)-**
ε–oxycaproyl unit	**Cap**	**-(O-CH_2-CH_2-CH_2-CH_2-CH_2-CO-)-**
		ε δ γ β α

Polyester chain structures may be engineered by appropriate combination of monomers, initiators (anionic, cationic, or coordination) and proper selection of conditions of polymerization processes (temperature, solvent) [3-5].

Nature of initiators and polymerization conditions influence structures of obtained polyesters also by changing the role of transesterification reactions accompanying polymerization and/or copolymerization. The transesterification processes include reactions between different polyester chains (intermolecular transesterification) or within a polyester chain (intramolecular transesterification – back biting) [6].

HR-NMR spectroscopy is often a very useful tool for monitoring of processes engineering structures of polyester chains.

1. Microstructure of polylactide

Since 1975 various research groups investigated microstructure of polylactide [7,8]. Vert was the first who described 'predominantly isotactic' structure as a consequence of nonselective polymerization of racemic lactide without transesterification [9]. There are two equivalent asymmetric carbon atoms in lactide molecules and therefore there are racemic and meso forms of this monomer. Various polyester structures obtained from lactide monomer are shown in Scheme 1.

Scheme 1:. Lactide homopolymers -$(LL)_n$*-

optically inactive	[R]=[S]	...RRRR.. +...SSSS...	isotactic	..iiiii...
optically inactive	[R]=[S]	...RSRSRSRS....	syndiotactic	...sssss...
optically inactive	[R]=[S]	...RRSSSRSRR....	atactic	...isiissi...
optically inactive	[R]=[S]	...RRSSRRSSRR...	disyndiotactic	...isisisi...
optically inactive	[R]=[S]	...RRSSRRSSRR...	„predominantly isotactic"	...isiiisiii... without ss sequences
optically inactive	meso	..RSSRSRSRRS...	„predominantly syndiotatctic"	...sissssis... without ii sequences
optically active	[S]=0 lub [R]=0	...RRRR...lub ..SSSS...	isotactic	...iiiiiii...
optically active	[R]≠[S]	...RRRRSSRSSS	intermediate structures	

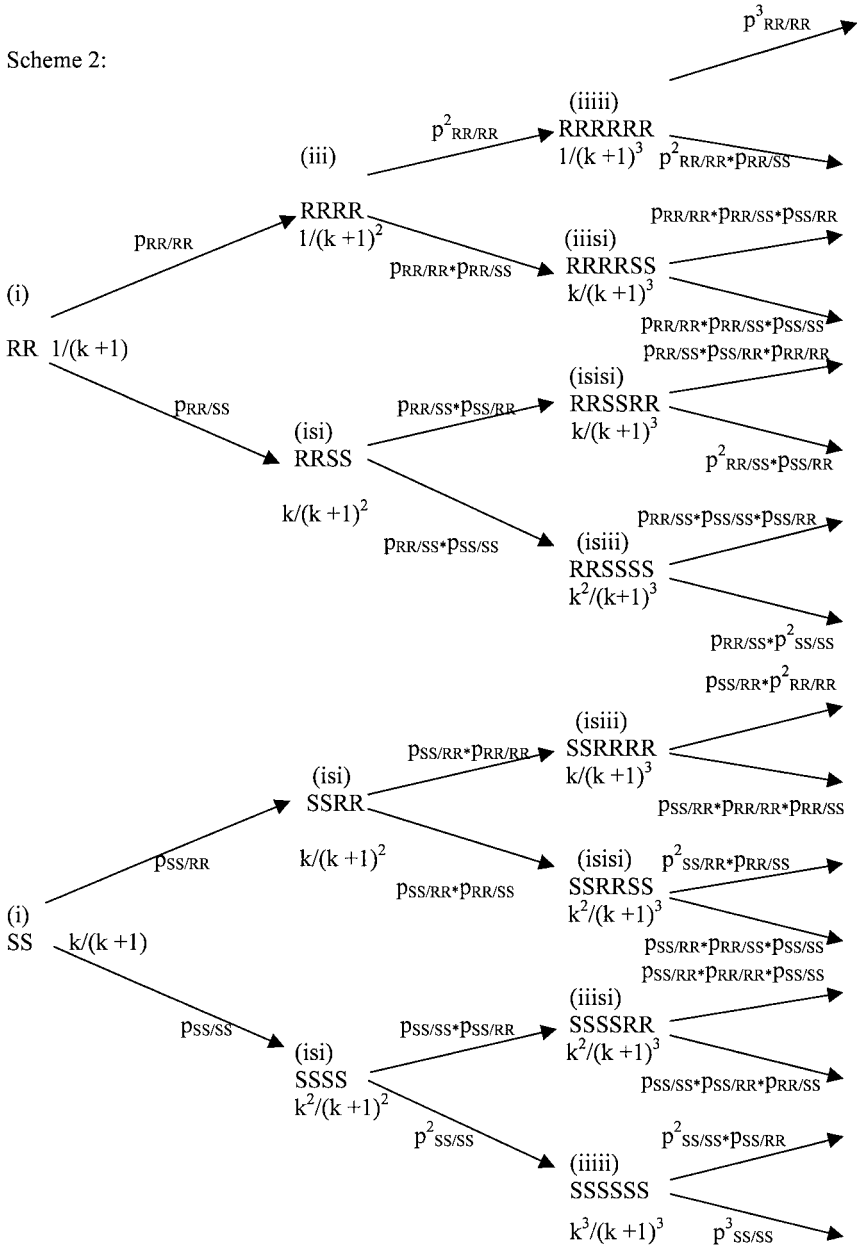
In the polymerization of lactide in each propagation step the addition to the growing chain of a pair of asymmetric equivalent carbon atoms does occur. Scheme 2 shows the statistical model of propagation in the polymerization of lactide.

When $p_{RR/SS}+p_{SS/RR}=1$ the propagation occurs by pair addition according to Bernoullian statistics. Value k denotes the ratio [SS]/[RR]. In non stereoselective polymerization without transesterification theoretical values of all n-ads intensities may be calculated [10] (e.g. tetrads): $(iii)=(2k^2-k+2)/2(k+1)^2$; $(isi) = k/(k+1)^2$; $(iis) = (sii) = (sis) = k/2(k+1)^2$.

In such conditions polymer with 'predominantly isotactic' structure is formed from racemic monomer, from non-equivalent mixtures of enantiomers – partly isotactic polymers may be obtained in which with increase of optical activity of monomers isotacticity increases. Spectra of polylactides obtained in the presence of initiators inducing transesterification are more complex and such structures cannot be described by pair addition Bernoullian statistics. In the polymerization of racemic mixture with high transesterification near atactic structure of polyester chain is obtained.

In polylactide chains obtained from racemic mixture by pair addition without transesterification formation of ss segments is forbidden that's why 3 triads, 5 tetrads, 7 pentads and 11 hexads are present.

Scheme 2:


(i)
RR 1/(k +1)
pRR/RR
RRRR
1/(k +1)2
pRR/SS
(isi)
RRSS
k/(k +1)2
(iii)
p2RR/RR
(iiiii)
RRRRRR
1/(k +1)3
p3RR/RR
p2RR/RR*pRR/SS
pRR/RR*pRR/SS
(iiisi)
RRRRSS
k/(k +1)3
pRR/RR*pRR/SS*pSS/RR
pRR/RR*pRR/SS*pSS/SS
pRR/SS*pSS/RR
(isisi)
RRSSRR
k/(k +1)3
pRR/SS*pSS/RR
p2RR/SS*pSS/RR
pRR/SS*pSS/SS
(isiii)
RRSSSS
k2/(k+1)3
pRR/SS*pSS/SS*pSS/RR
pRR/SS*p2SS/SS
(i)
SS k/(k +1)
pSS/RR
SSRR
k/(k +1)2
pSS/SS
(isi)
SSSS
k2/(k +1)2
pSS/RR*pRR/RR
(isiii)
SSRRRR
k/(k +1)3
pSS/RR*p2RR/RR
pSS/RR*pRR/RR*pRR/SS
pSS/RR*pRR/SS
(isisi)
SSRRSS
k2/(k +1)3
p2SS/RR*pRR/SS
pSS/RR*pRR/SS*pSS/SS
pSS/SS*pSS/RR
(iiisi)
SSSSRR
k2/(k +1)3
pSS/RR*pRR/RR*pSS/SS
pSS/SS*pSS/RR*pRR/SS
p2SS/SS
(iiiii)
SSSSSS
k3/(k +1)3
p2SS/SS*pSS/RR
p3SS/SS

To the contrary, in poly(meso-lactide) the ii connections are not possible. Only two tetrads are identical in poly (meso-lactide) and poly(racemic-lactide) chains, i.e. isi and sis. However, their intensities in the spectra of respective polylactides are different. Correlation of methine range in carbon NMR spectra and homodecoupled proton spectra by heteronuclear chemical shift correlation NMR method allows to assign the signals in H-1 (homodecoupled) NMR spectra to pentads and C-13 NMR to tetrads [11].

In the case of stereoselective polymerization of racemic lactide it is reasonable to assume that the probabilities of enantiomer addition to the growing chain terminated with the same enantiomer are equal ($p_{RR/RR}=p_{SS/SS}=p_1$). For such process the probabilities of the enantiomer addition to the growing chain terminated with opposite enantiomers are equal too ($p_{RR/SS}=p_{SS/RR}=p_2$).

Values of intensity of all individual sequences (i.e. hexads) could be calculated using following expressions[12]:

$(iiiii) = p_1^3 + 0{,}5p_1^2p_2$

$(iiis) = (siii) = (iisii) = 0{,}5p_1^2p_2$

$(iisi) = (isii) = 0{,}5p_1^2p_2 + 0{,}5\ p_1p_2^2$

$(iisis) = (siis) = (sisii) = 0{,}5\ p_1p_2^2$

$(isisi) = 0{,}5p_1p_2^2 + 0{,}5p_2^3$

$(sisis) = 0{,}5p_2^3$

Probability coefficients p_1 and p_2 could be determined using intensities of signals in NMR spectra.

Structures of polylactide chains obtained from racemic monomer with various p values are shown in Scheme 3.

Scheme 3:

R,R+S,S(p_1=1,p_2=0) ⇒...SSSSSS...+...RRRRRRR... isotactic

R,R + S,S($p_1 = p_2 = 0{,}5$)⇒ ...SSSRRRSSSSRRR... „predominantly isotactic"

R,R + S,S(p_1= 0, p_2 = 1)⇒ ...SSRRSSRRSSRR.... disyndiotactic

The average length of isotactic blocks (counting on lactyl unit) in fully disyndiotactic structure equals 2 but in 'predominantly isotactic' structure equals 4. In the spectrum of

completely disyndiotactic polymer two lines with equal intensities are expected on the basis equations corresponding to hexads and tetrads. In C-13 NMR spectrum of poly(racemic-lactide) obtained using lithium t-butoxide as an initiator increase of two lines are observed in range of carbonyl and methine signals. Calculated coefficient probability indicates preference of alternating SS and RR enantiomer additions to the growing chains. This preference results in an increase of disyndiotactic structures in polyester. Influence of reaction time and temperature on chain microstructure in the stereoselective polymerization of racemic lactide is shown in Table 1.

Table 1. Polymerization of racemic lactide using lithium t-butoxide as initiator.

No	Temp. (C^0)	Time (min)	Y (%)	M_n (x 10^{-3})	MWD	Tetrads intensities (%)					L_i	p_2
						ssi	sss	isi	iss	sis, iis, sii, iii		
disyndiotactic structure						0	0	50	0	50	2,00	1,00
1	-20	5,0	35	35	1,4	0	0	47	0	53	2,13	0,94
2	-20	17,0	58	41	1,55	5	2	36	5	52		
3	-20	60,0	74	45	1,6	7	4	31	7	51		
4	0	3,0	45	26	1,4	0	0	46	0	54	2,17	0,92
5	0	10,0	74	42	1,6	6	2	37	6	49		
6	+20	0,5	35	24	1,4	0	0	45	0	55	2,22	0,90
7	+20	1,0	55	41	1,5	3	0	39	3	55		
8	+20	40,0	78	50	1,6	9	6	31	9	45		
'predominantly isotactic' structure						0	0	25	0	75	4,00	0,50

In polymerization of racemic lactide stereoselection was achieved also using butyllithium and butylmagnesium for initiation [13,14]. In the case of butyllithium, low initiator concentration yields partly disyndiotactic structure without ss segments. Increase of initiator concentration was manifested by the change of macromolecular chain structure by generation of ss segments. The disyndiotacticity of polylactide chains obtained using butylmagnesium was smaller in comparison with polylactide obtained with lithium initiators (p_2=0.63) but transesterification was absent even for high initiator concentrations or at elevated polymerization temperature (up to 80 ^oC formation of ss sequences wasn't observed in NMR spectra).

2. Microstructure of lactide-ε-caprolactone copolymer

The possible polyester structures of copolymers obtained from lactide and ε-caprolactone are shown in Scheme 4.

Scheme 4:

-(LL)$_n$*-(Cap)$_m$	block AB type
-(LL)$_n$* -(Cap)$_m$-	block with various lengths of blocks n>2, m>2
-(LL)$_n$*-[(Cap)$_m$-L-(Cap)$_p$-]$_q$-	block with various length of blocks n>2, m>2 containing CapLCap sequences
-(LL)$_n$*-(Cap)$_m$-	statistical; n and m dependent on comonomer ratio
-(LL)$_n$*-[(Cap)$_m$-L-(Cap)$_p$-]$_q$-	statistical; containing CapLCap sequences
-(LL)$_n$*-(Cap)$_m$-	alternate n=m=1

Assignment of spectral lines to appropriate sequences was made on the basis of following analyses [15]:

1. Comparison of homopolymers spectra with the spectra of statistical copolymer
2. Comparison of copolymer spectra obtained by initiator assuring absence of the second mode of transesterification ($Al(acac)_3$) with various comonomer ratios
3. Comparison of copolymer spectra obtained by initiator inducing second mode of transesterification with various comonomer ratios
4. Application an additivity rules of C-13 chemical shifts to assignment of CapLLCap triad
5. Comparison with model compound with purpose to assign CapLCap sequences.

Complete description of copolymerization of lactones requires quantitative analysis of transesterification [16,17]. In the case of copolymerization with only the first mode of transesterification the yield of transesterification T_1 could be calculated from equation **(1),** where $\mathcal{L}^T_{LL}=(k+1)/k$ denotes the average length of lactidyl blocks in completely random chains and $\mathcal{L}^r_{LL}$ indicates length of blocks calculated from reactivity coefficient.

$$T_I = \frac{\mathcal{L}^r_{LL} - \mathcal{L}^e_{LL}}{\mathcal{L}^r_{LL} - \mathcal{L}^T_{LL}} \qquad (1)$$

When second mode of transesterification T_{II} operates during copolymerization process (cleavage of lactidyl unit), the yield of this transesterification may be calculated from equation **(2)**, where [CapLCap] denotes concentration of CapLCap sequences (experimentally determined) in copolymer chain and $[CapLCap]_R$ – concentration of CapLCap sequences in completely random chains described by the relation **(3)**, k' denotes the ratio [Cap]/[L].

$$T_{II}=[CapLCap]/[CapLCap]_R \qquad (2) \qquad [CapLCap]_R=k'^2/(k'+1)^3 \qquad (3)$$

The good example of the first mode of transesterification in the lactide-ε-caprolactone copolymerization is the reaction in the presence of $Al(acac)_3$. Copolymerizations were conducted to low conversion (ca 5 mol-%) and theoretical average length of blocks on the basis of the reactivity ratios were calculated L^r_{LL} and L^r_{Cap}. Values L^r_{LL} and L^r_{Cap} are markedly higher than experimental average length of blocks and calculated T_I coefficients are nearly 1. Above findings indicate strong transesterification processes in the first step of copolymerization reaction although the lines from CapLCap sequences (T_{II}=0) are not observed in NMR spectra. When the structure of the obtained polyester was estimated at progressing conversion, a sudden decrease of the average length of lactidyl blocks accompanied by a slow increase of caproyl blocks, was observed (Figure.1).

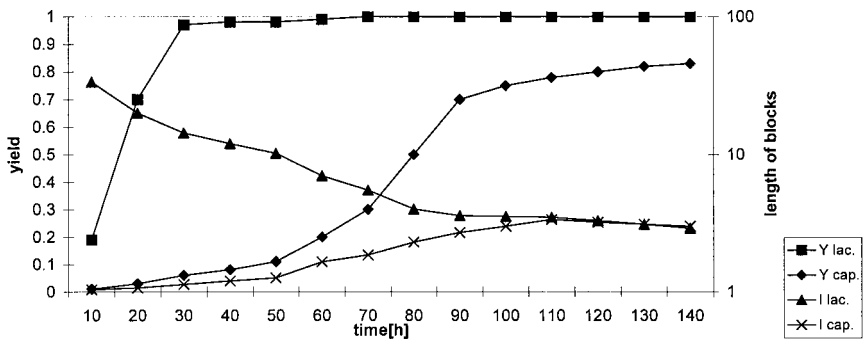


Figure 1. Relations between lengths of blocks (lactydyl blocks $l_{lac.}$ and caproyl blocks l_{cap}) and yields of lactide $Y_{lac.}$ and caprolactone $Y_{cap.}$ in the copolymerization in the presence of $Al(acac)_3$

The average length of lactidyl blocks decreases even after complete conversion of lactide in reaction mixture. Because CapLCap sequence doesn't appear till the end of the reaction, the decrease of lactide block length must be due to the first mode of transesterification.

Effects of the second mode of transesterification are well seen in copolymerization of lactide with ε-caprolactone in the presence of zinc or lithium initiators (Figure 2).

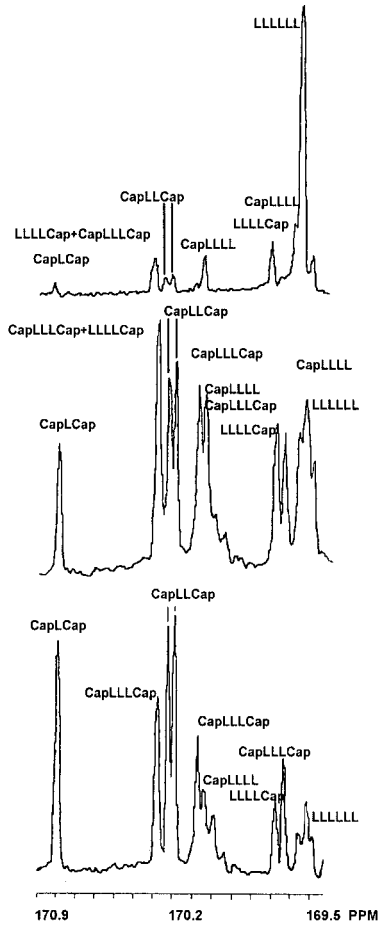


Figure 2. C-13 NMR spectra of L,L-lactide / ε-caprolactone copolymer (carbonyl region of lactide) a) multiblock copolymer with small contents of CapLCap sequences , b and c) alternating copolymers with high contents of CapLCap sequences

The influence of the structure of lactide on transesterification processes in copolymerization of lactide with ε-caprolactone was investigated [18]. In all analyzed copolymerizations a considarably decrease in the caproyl and lactidyl block length and accompanying increase in randomization of the polyester chains were found when racemic lactide was used instead of S,S-lactide. The transesterification was related to the attack of an active caproyl chain end on the lactydyl blocks. Using racemic lactide as comonomer allowed observation of other transesterification reaction involving the attack of an active lactyl center ~L* on lactydyl blocks of the already formed chains.

3. Microstructure of glycolide/ε-caprolactone copolymers

Variety of polyester structures that could be obtained from glycolide and ε-caprolactone is shown on Scheme 5.

Scheme 5:

-(GG)$_n$-(Cap)$_m$-	block AB type
-(GG)$_n$-(Cap)$_m$-	block with various lengths of blocks n>2, m>2
-(GG)$_n$-[(Cap)$_m$-G-(Cap)$_p$]$_q$-	block with various length of blocks n>2, m>2 containing CapGCap sequences
-(GG)$_n$-(Cap)$_m$	statistical; n and m dependent on comonomer ratio
-(GG)$_n$-[(Cap)$_m$-G-(Cap)$_p$]$_q$-	statistical or multiblock; containing CapGCap sequences
-(GG)$_n$-(Cap-G-Cap)$_p$-(Cap)$_q$	segmental
-(GG)$_n$-(Cap)$_m$-	alternate n=m=1

Similar procedure as in the case of lactide/caprolactone copolymers was used for analysis of NMR spectra of glycolide/caprolactone copolymers [19]. In C-13 NMR spectra all signals due to methylene and carbonyl carbon appeared to be sensitive to the copolymer microstructure. The proton spectra of the glycolide/ε-caprolactone copolymers provide much more information than the H-1 NMR spectra of lactide/caprolactone copolymers (Figure 3).

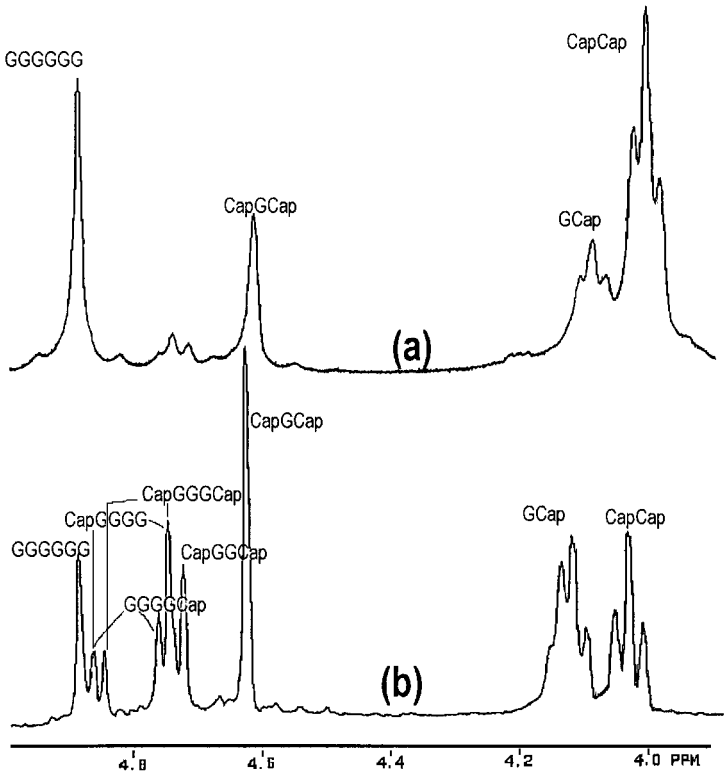


Figure 3. H-1 NMR spectra of poly(glycolide-co-ε-caprolactone) obtained using $Al(acac)_3$ as initiator (a) at 100°C- segmental structure (b) at 150°C –random structure

The H-1 NMR spectra allow not only for determination of the copolymer compositions but also for analysis of the microstructure from signals of ε-methylene protons of ε-oxycaproyl unit and from glycolide methylene protons. Average length of blocks and transesterification $T_{II}=[CapGCap]/[CapGCap]_R$ can be calculated from the proton NMR spectra, where [CapGCap] denotes experimental concentration of CapGCap sequences in copolymer and $[CapGCap]_R$ concentration in completely random chain $[CapGCap]_R=k'^2/(k'+1)^3$.

4. Microstructure of glycolide/lactide copolymers

Structures of polyesters obtained from lactide and glycolide are shown below in Scheme 6.

Scheme 6

- $(LL)_n$*-$(GG)_m$	block AB type
-$(LL)_n$*-$(GG)_m$-	block with various lengths of blocks n>2, m>2
-[$(L)_r$-G-$(LL)_n$*]$_s$-[$(GG)_m$-L-$(G)_p$]$_q$-	block with various length of blocks n>2, m>2 containing GLG and/or LGL sequences
-$(LL)_n$*-$(GG)_m$-	statistical, n and m dependent on comonomer ratio
-[$(L)_r$-G-$(LL)_n$*]$_s$-[$(GG)_m$-L-$(G)_p$]$_q$-	statistical containing GLG and/or LGL sequences
-$(LL)_n$*-$(GG)_m$-	alternate n=m=1

The structural analysis of poly(glycolide-co-lactide) microstructure was based on C-13 and H-1 NMR spectroscopy [20]. In the carbonyl region of C-13 spectrum the simplest diad sequences were observed and GLG sequences like in copolymerization with transesterification. The analysis of H-1 NMR spectra was performed using methylene group signal of glycolide. This approach allows monitoring longer sequences and also a sequence LGL from transesterification.

For quantitative evaluation of the role of transestrification the following expressions could be used for LGL sand GLG equences generated by transesterification:

$T_{II\,(LGL)} = [LGL]/[LGL]_R$ **(4)** $[LGL]_R = k/(k+1)^3$ **(5)**

$T_{II\,(GLG)} = [GLG]/[GLG]_R$ **(6)** $[GLG]_R = k^2/(k+1)^3$ **(7)**

References

1. D.F. Williams, „Definitions in Biomateerials" w Progress in Biomedical Engineering, **4**, Elsevier, Amsterdam, The Netherlands (1987)
2. L.L. Hench, E.C. Ethridge, „Biomaterials, an Interfacial Approach", Academic Press, New York, USA, (1982)

4. H.R.Kricheldorf, I. Kreiser, *Makromol.Chem.***188**, 1861, (1987)
5. N.Ropson, Ph.Dubois, R.Jerome, Ph.Teyssie, *Macromolecules,* **27**, 5950, (1994)
6. H.R.Kricheldorf, I. Kreiser, *J.Macromol.Sci. Chem.***A24**, 1345, (1987)
7. E.Lillie, R.C.Schulz , *Makromol.Chem.***176**, 1901, (1975)
8. A.Schindler, D.J.Harper, *J.Polym.Sci.Polym.Lett.Ed.*.**14**, 705, (1976)
9. F.Chabot, M.Vert, *Polymer,* **24**, 53, (1983)
10. M. Bero, J. Kasperczyk, Z. Jedliński, *Makromol. Chem.* **191**, 2287 (1990)
11. Kasperczyk J. *Polymer* **40**, 5455 (1999)
12. Kasperczyk J. *Macromolecules* **28**, 3937 (1995)
13. Kasperczyk J., Bero M. *Polymer* **41**,391(2000)
14. Bero M., Dobrzyński P., Kasperczyk J. *J.Polym.Sci.,Part A*,**37(22),**4038 (1999)
15. Kasperczyk J., Bero M. *Makromol. Chem.* **192**, 1777(1991)
16. M. Bero, J. Kasperczyk, G. Adamus, *Makromol. Chem.* **194**, 907 (1993)
17. Kasperczyk J., Bero M. *Makromol. Chem.* **194**, 913(1993)
18. Bero M., Kasperczyk J. *Macromol.Chem.Phys.* **197**,3251 (1996)
19. Kasperczyk J. *Macromol. Chem. Phys.* **200**, 903(1999)
20. Kasperczyk J. *Polymer* **37**, 201 (1996)