Polymers for Multifunctional Textiles

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Summary: Biodegradable P[(R,S)-3HB-co-LA] and P[(R,S)-3HB-co-CL] copolyesters for special medical application, i.e. as scaffolds for cartilage and soft tissue engineering, were synthesized by transesterification of the corresponding homopolymers, i.e. a-PHB and poly(L-lactide) or a-PHB and poly(e-caprolactone), respectively. The carried out biological studies emphasized the biocompatibility of the copolymer PHBV/a-PHB, in for its using in the manufacturing of the haemostatical dressings.

Keywords: biodegradable; biomaterials; biopolymers; chain; implant

Introduction

Poly(L-lactide) (PLLA) and poly (e-caprolactone) (PCL) are considered as very promising candidates for the manufacture of biomaterials. Atactic poly(3-hydroxybutyrate), a-PHB, has previously been selected as a blend component with microbial PHB and poly(3-hydroxybutyrate-co-3-hydroxyvalerate). P(3-HB-co-3-HV), PLLA and poly(e-caprolactone) (PCL), with the aim of improving their biodegradability and mechanical properties like flexibility and impact resistance, which are of importance in the development of new biomaterials for temporary soft tissue applications. Various catalytic systems have been tested for the synthesis of copolymers containing structural segments of atactic PHB via ring opening copolymerization of racemic b-butyrolactone. The synthesis of copolyesters by transesterification is usually carried out in the molten state, starting from homopolyesters derived from dicarboxylic acids and dialcohols.

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Experimental Issues

- PCL ($Mn_{SEC} = 83\,500$, $Mw/Mn = 1.6$) and Poly(L-lactide) ($Mn_{SEC} = 82\,000$, $Mw/Mn = 1.7$)

– were purified by reprecipitation in hexane from chloroform solution;
– were filtered, washed with hexane, and dried under vacuum at room temperature.
– Poly[(R,S)-3-hydroxybutyrate)], a-PHB ($Mn_{SEC} = 20\,000$, $Mw/Mn = 1.6$), was synthesized by bulk polymerization of (R,S)-b-butyrolactone at room temperature, using KOH/18-crown-6 complex as initiator.
– The polymerization was monitored by Fourier transform infrared (FT-IR) spectroscopy and the monomer-to-polymer conversion was measured using the intensities of the carbonyl stretching vibrational bands of the lactone monomer (1815 cm^{-1}) and of the polyester formed (1760 cm^{-1}). ^{13}C NMR and ^{1}H NMR spectra showed that the polymer obtained was atactic, while DSC analysis confirmed its amorphous character.
– 4-Toluenesulfonic acid monohydrate, was of the highest purity commercially available and was used without further purification.

2-(4-Hydroxyphenilazo) benzoic acid (HABA) and solvents were used as supplied.

Preparation of Copolyesters

Copolyesters were synthesized by transesterification of the corresponding homopolymers in solution, in the presence of 4-toluenesulfonic acid monohydrate (Scheme 1).

Analyses

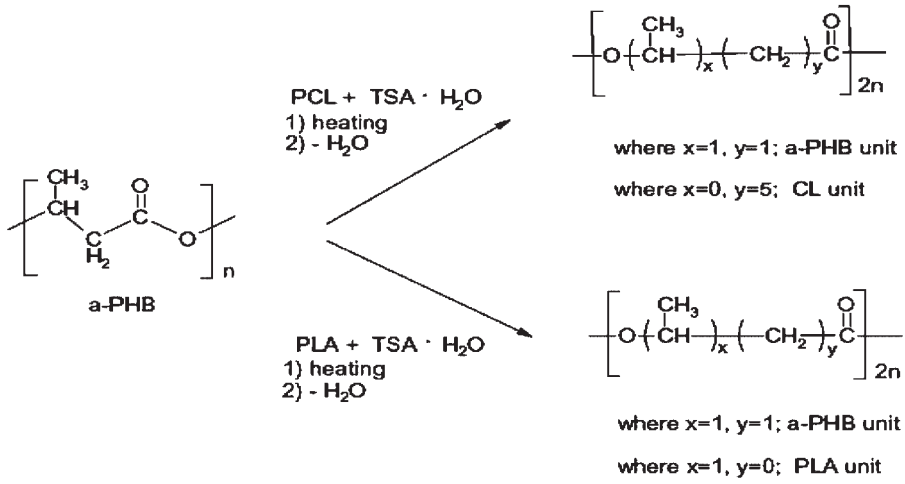
NMR Analysis

^{1}H NMR spectra of (P[(R,S)-3HB-co-LA]) copolyesters: - recorded at room temperature in $CDCl_3$; - tetramethylsilane (TMS) as internal standard using a Varian VCR-300 spectrometer with a 4499 Hz spectral width, acquisition time 1.998 s, and 64 repetitions.

^{1}HNMR analyses of P[(R,S)-3HB-co-CL] copolyesters: - performed at 278° C; - Varian UNITY INOVA spectrometer operating at 500 MHz, using deuterated chloroform as solvent (40 mg/mL) and TMS as internal standard. - Spectra were acquired with a spectral width of 6000 Hz and processed using WINNMR (Bruker). The 500MHz 1H NMR spectra of P[(R,S)-3HB-co-CL]; - recorded before and after the addition of D_2O and shaking, to confirm the assignment.

SEC Analysis

SEC analyses were carried out in THF with a Waters 515 HPLC apparatus, equipped with four Ultrastyragel HR columns (in the order HR4, HR3, HR2 and HR1) connected in series, using a Waters R401 differential refractive index detector. A polymer solution (100 mL, 1 mg/mL) was injected and eluted at a flow rate of 1 mL/min. The fractionation of copolyesters samples was performed by collecting 10 drops (about 0.24 mL) for each fraction. Sample preparation for MALDI-TOFMS analysis 2-(4-Hydroxyphenilazo) benzoic acid (HABA) (0.1Min THF) was used as matrix. The unfractionated copolymer samples were dissolved in THF and a concentration of 2–3 mg/mL was used. SEC fractions were dissolved in THF (from 20 up to 100 mL, according to the fraction number). Equal volumes of copolyester sample solutions and matrix solution were mixed in order to obtain ratios of 1:1, 1:2, and 1:3 (sample/ matrix, v/v). MALDI-TOFMS analysis MALDI-TOF mass spectra were recorded in linear and/or in reflector modes. A Voyager-DE STR (PerSeptive Biosystems) mass spectrometer equipped with a nitrogen laser, emitting at 337nm with 3-ns pulse width, and working in positive ion mode, was used.



Scheme 1.
Synthetic pathway of P[(R,S)-3HB-co-LA] and P[(R,S)-3HB-co-CL] copolyesters.

Results and Discussion

The compositions of P[(R,S)-3HB-co-LA] copolyesters were estimated by the area integration of the ^{1}H NMR signals of the protons of methyl groups CH_3- at 1.28 ppm (in (R,S)-3-HB units) and at 1.58 ppm (in LA units).

Molar Mass Determination

The average molar masses of the samples were determined by off-line SEC/MALDI-TOFMS. Accordingly, both series of polydisperse samples were fractionated by SEC and the collected fractions were analyzed by MALDITOFMS. Thus, mass spectra of nearly monodisperse samples with a narrow molar mass distribution were acquired. The resulting calibration curve enabled the computation of the average molar masses (MM) and molar mass distributions (MMD) of the unfractionated samples directly from their SEC traces. Figure 1(a) shows the SEC trace of a P[(R,S)-3HB-co-LA] (10:90) copolyester sample 1 together with the corresponding SEC/MALDI-TOFMS calibration curve obtained by the fractionation of this sample and the MALDI analysis of the narrow SEC fractions collected (SEC/MALDI-TOFMS method). The same calibration curve was also used to determine the average molar masses of unfractionated sample.

In the mass spectra of low molar mass fractions, ions corresponding to the HABA matrix partly covered the ions belonging to the selected fractions. The highresolution MALDI-TOF mass spectra obtained for the collected narrow fractions were useful for the structural characterization of these samples.

Structural Characterization of P[(R,S)-3HB-co-LA] Copolyesters by MALDI-TOFMS

Figure 2 shows the MALDI-TOF mass spectrum, acquired in reflector mode, of the P[(R,S)-3HB-co-LA] (10:90) copolyester. It extends up to m/z 5500 and it shows a distribution of singly charged sodium adduct ions in clusters discriminated by different degrees of oligomerization and compositions. Ions with differences of 72, 86 and 14 Da are observed. These values correspond, respectively, to the masses of lactic acid and hydroxybutyrate units and also to the difference in mass of 3-hydroxybutyrate and lactic acid units (14 Da). All the ions can be identified as sodium-cationized linear copolyester chains terminated with carboxyl and hydroxyl end groups.

Figures 3(a)–(c) -the MALDI-TOF mass spectra, recorded in linear mode, of selected fractions of P[(R,S)-3HBco-LA] (10:90) copolyester), collected at (a) 25.75, (b) 28.43, and (c) 30.14 mL, respectively. This spectrum allows the assignment of each ion to a specific oligomer, and the identification of the structure of the copolymer and the end groups of the individual chains. Figure 4 displays the high-resolution MALDI-TOF mass spectrum, acquired in reflector mode, of a low molar mass fraction collected for a P[(R,S)-3HB-co-LA] copolyester, and a spectral expansion in the mass range m/z 1600–1850. In principle, NMR can be used to determine the sequence distribution. In practice, however, NMR peak assignment at the triad level is needed. The latter is rather difficult and a- PHB tacticity reasons led us to omit this type of analysis.

Structural Characterization of P[(R,S)-3HB-co-CL] Copolyesters by MALDI-TOFMS

Figure 5 displays the MALDI-TOF mass spectrum of unfractionated P[(R,S)-3HB-co-CL] (44:56) copolyester that contains about 220 ions. The ion signal intensity falls starting from low m/z values to high values, until the signal reaches the baseline at approximately m/z 2600. The FWHM for each ion is 0.5 Th (or better), the isotopic peaks are resolved and the resolution is never less than 2500 (ranging between 2500 and 8000).

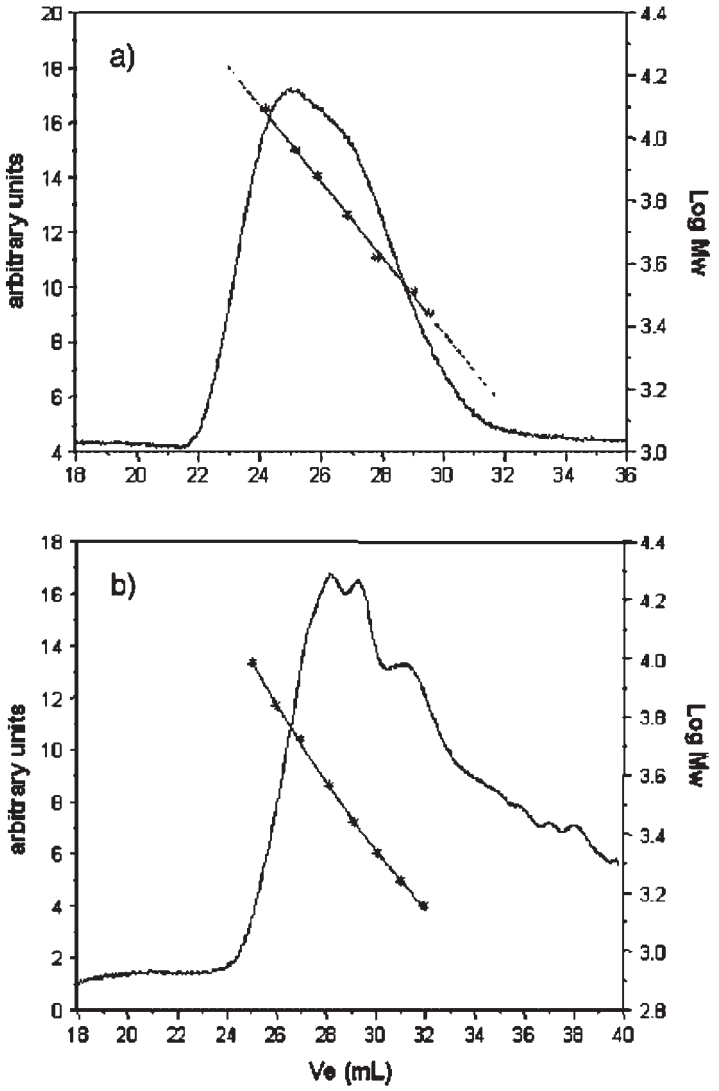


Figure 1.
(a) -the SEC trace of a P[(R,S)-3HB-co-LA] (10:90) copolyester sample together with the corresponding SEC/MALDI-TOFMS calibration curve obtained by the fractionation of this sample and the MALDI analysis of the narrow SEC fractions collected (SEC/MALDITOFMS method). (b). - the SEC trace and the SEC/MALDI calibration curve of P[(R,S)-3HB-co-CL] (35:65).

The most abundant ions, can be assigned to sodium-cationized linear copolyester chains terminated with hydroxyl and carboxyl end groups. The second type of ions (with lower signal intensity), can be assigned either to sodium-cationized cyclic oligomers or to linear copolyester chains terminated with tosyl (TSA) and carboxyl end groups. A third mass series, is due to the corresponding sodium salt of the linear hydroxyland carboxyl-terminated species. The mass regions in which hexamers, heptamers and octamers are detected are m/z 640–840, 730–950 and 810–1070,

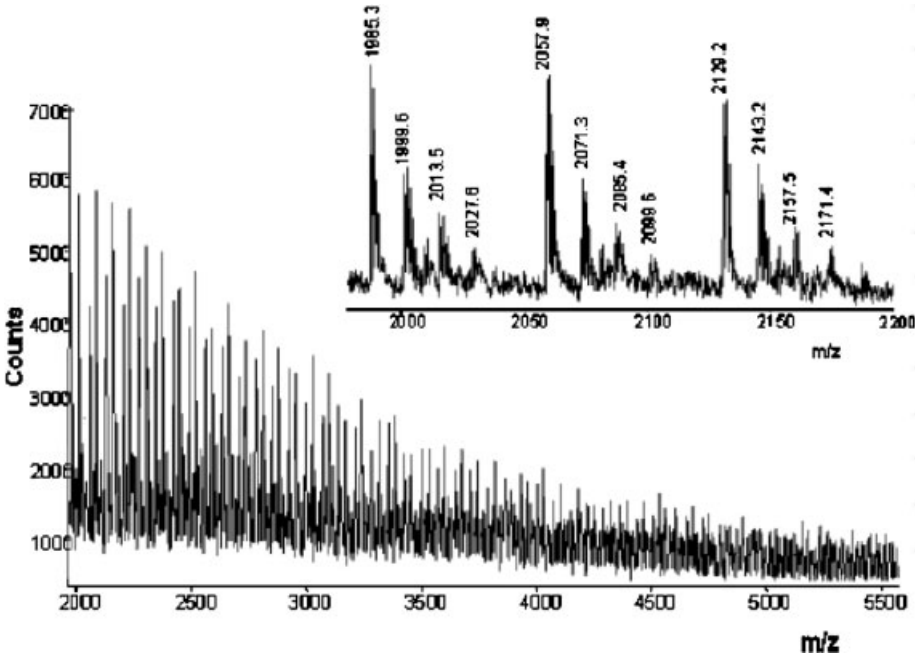


Figure 2.
MALDI-TOF mass spectrum in reflector mode of the unfractionated P[(R,S)-3HB-co-LA] (10:90) copolyester.

respectively, with a very strong overlap. Figure 6 shows the high-resolution MALDI-TOF mass spectrum of a relatively low MM fraction which was obtained by subjecting P[(R,S)-3HB-co-CL] (44:56) copolyester to chromatographic separation by SEC and collecting the fraction eluting at 32.27 mL.

The spectrum is unimodal and it is centred at m/z 1400. At a first sight, one may have the impression that all the ions belong to a single mass series. This is not the case. The lower portion of Fig. 6 reports a spectral expansion in the range m/z 1405–1480.

The MALDI mass spectrum of this copolyester fraction shows ions corresponding mainly to sodium-cationized linear copolyester chains terminated with hydroxyl and carboxyl end groups. The concurrent presence in the MALDI mass spectrum (Fig. 7) of P[(R,S)-3HB-co-CL] (35:65) linear and cyclic poly(e-caprolactone) homopolymer is probably due to the different reaction conditions applied for the synthesis of this sample. This spectrum is clearly bimodal. The figure also reports two expansions taken from regions R1 and R2, respectively, along with ion labels. The ions appearing in the first distribution at lower masses correspond to the sodium adducts of linear copolymer chains with different composition. The ions appearing in the second distribution at m/z 1848, 1962, 2076, 2190, 2304 and 2418 are due to sodium cationized cyclic species.

Bioresorbable products are used for *e*ach type of wound, when by skin and living tissues deterioration; local hemorrhage and/or fluid secretions occur in case of urgent interventions by naval road, air accidents, natural calamities. Requirements of the polymers in invasive medical devices application are: the ensuring of biological characteristics and of the products biocompatibility; the using of the biomaterials that correspond to the requirements imposed by 90/385/EC; the elaborating of the toxicological and physical – chemical methodology, according the demands of ISO 10993 and EN 30993.

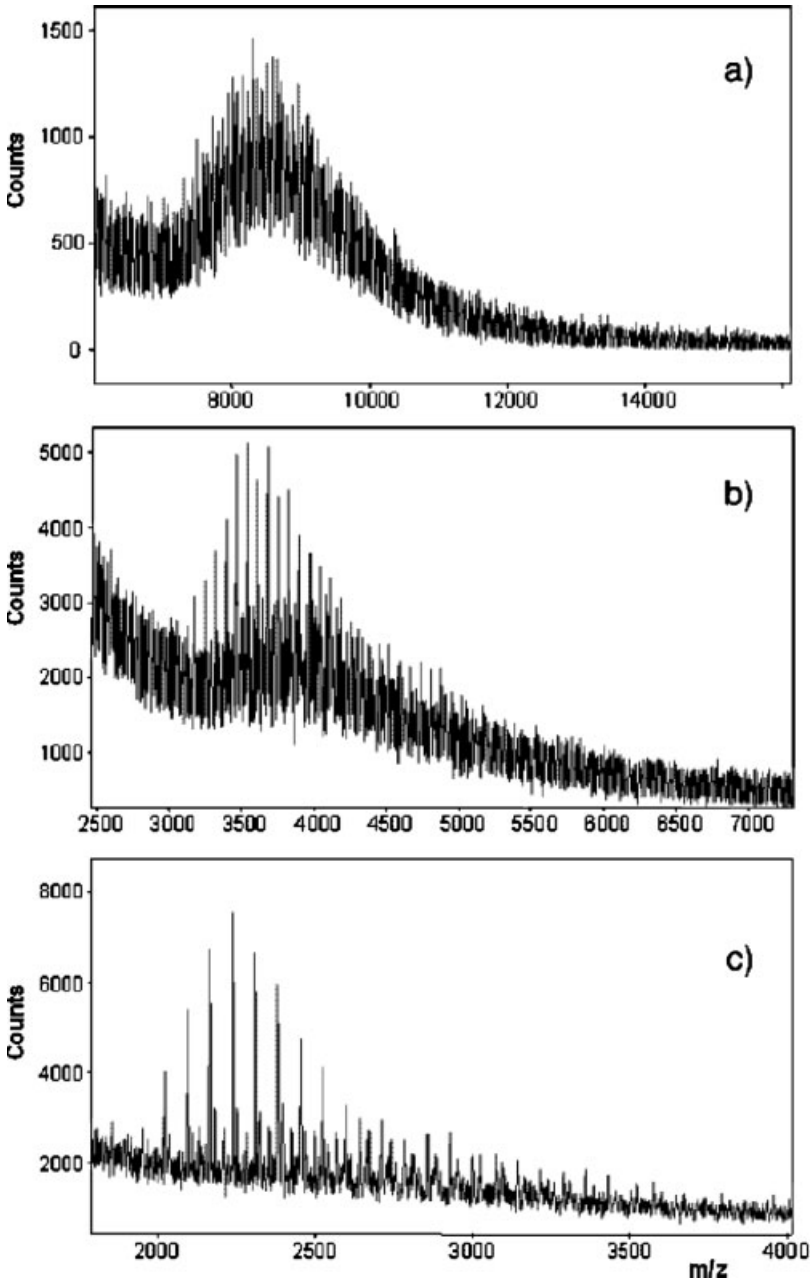


Figure 3.
(a)–3(c) - the MALDI-TOF mass spectra, recorded in linear mode, of selected fractions of P[(R,S)-3HBco-LA].

The biomedical and biofunctional characteristics are represented by: adequate conformity; flexibility; adaptability to wound topography - able to migrate while the host tissue slips; the maintenance of the resistance force - min 7 days; biocompatibility; inertia from the chemical point of view.

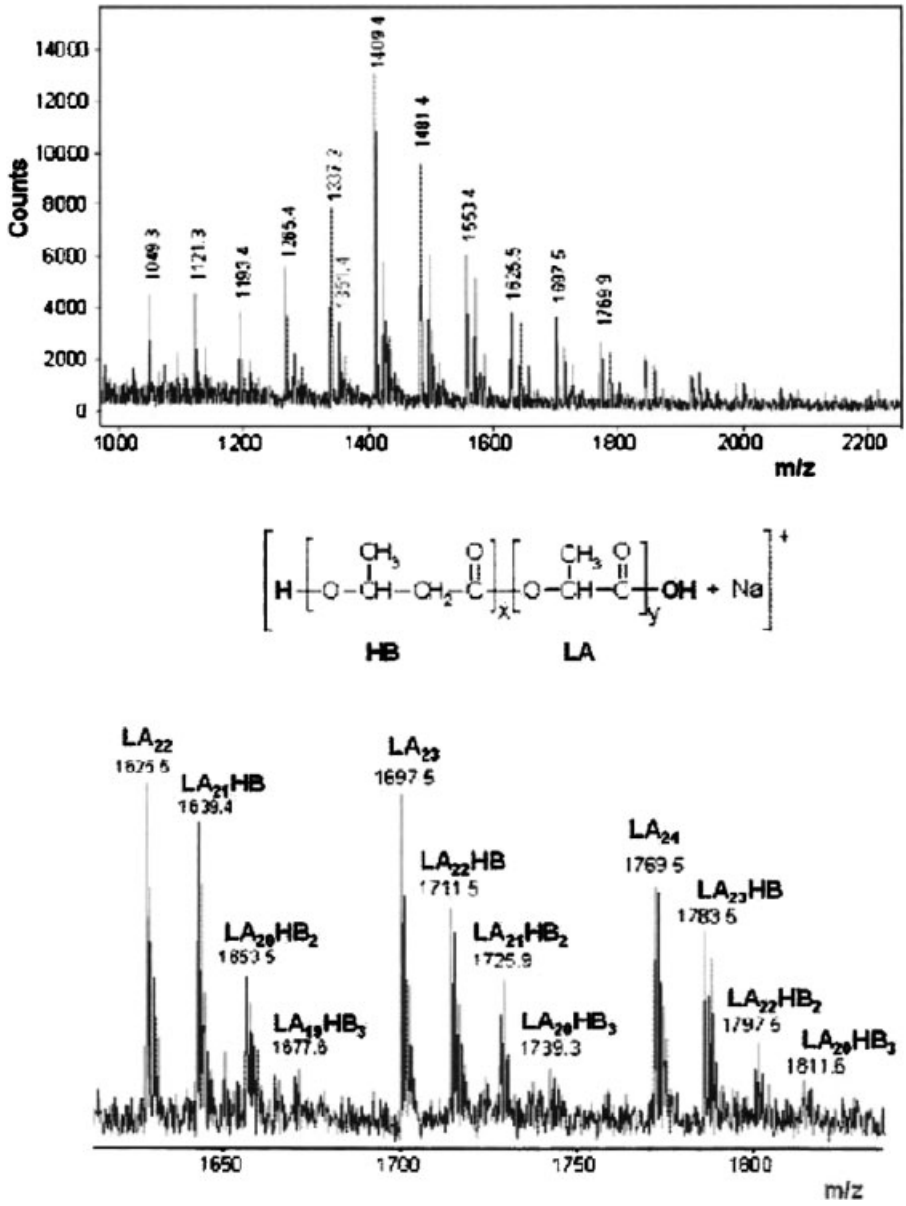


Figure 4.
the high-resolution MALDI-TOF mass spectrum, acquired in reflector mode, of a low molar mass fraction collected for a P[(R,S)-3HB-co-LA].

Obtaining of Spinning Solution and Rheologic Study

In view of identification the conditions for the obtaining of the spinning solution, there has been observed the influence of the polymer concentration and of temperature on the rheology of the polymer concentrated solutions. By preliminary tests there have been identified as optimal, the following parameters: temperature of dissolving: $75 \pm 5\,^{\circ}C$; duration of dissolving: 45 min. On the basis of this rheologic study there have

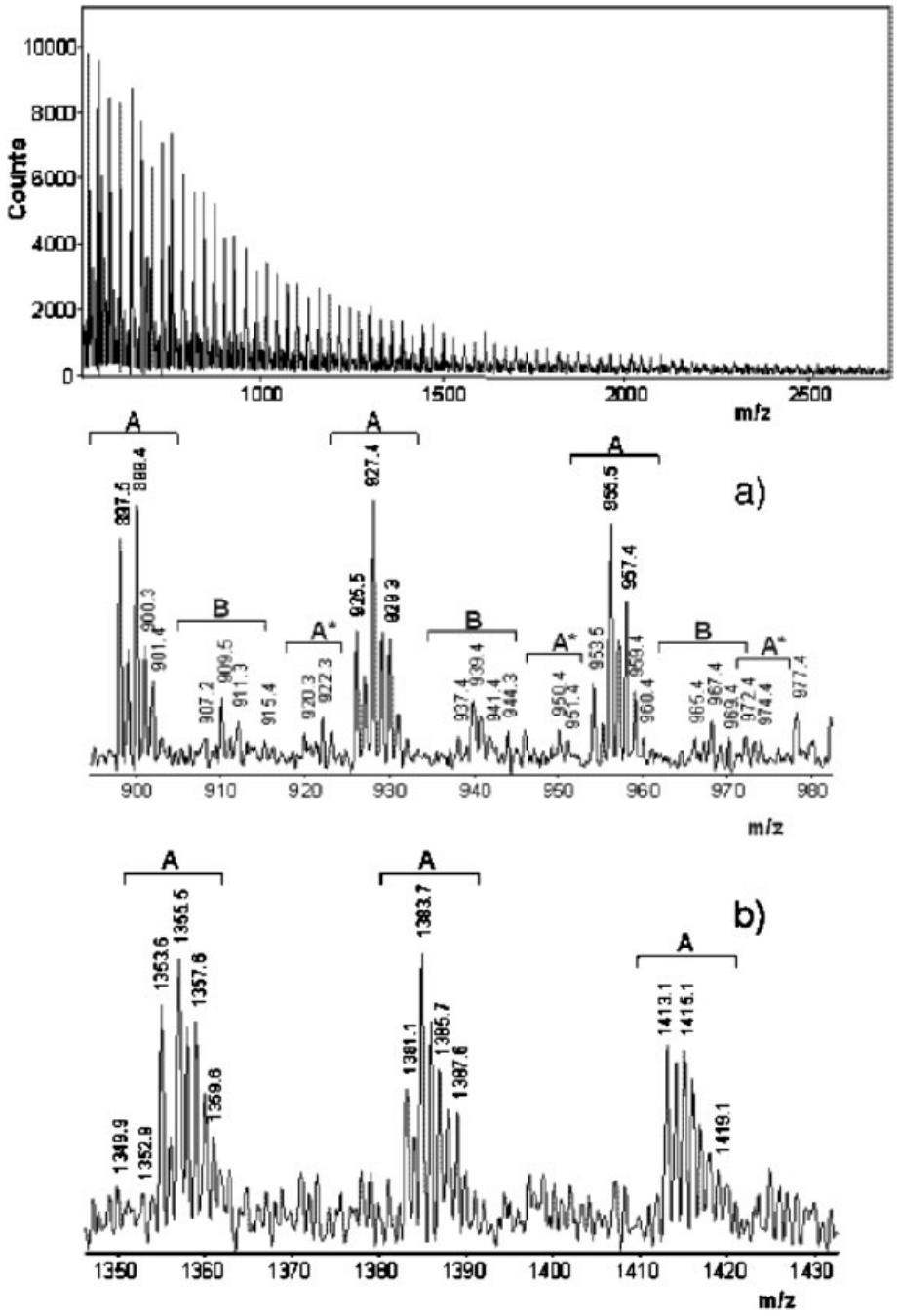


Figure 5.
(a) and 5(b) show two enlarged portions in the mass ranges m/z 895–980 and 1345–1430.

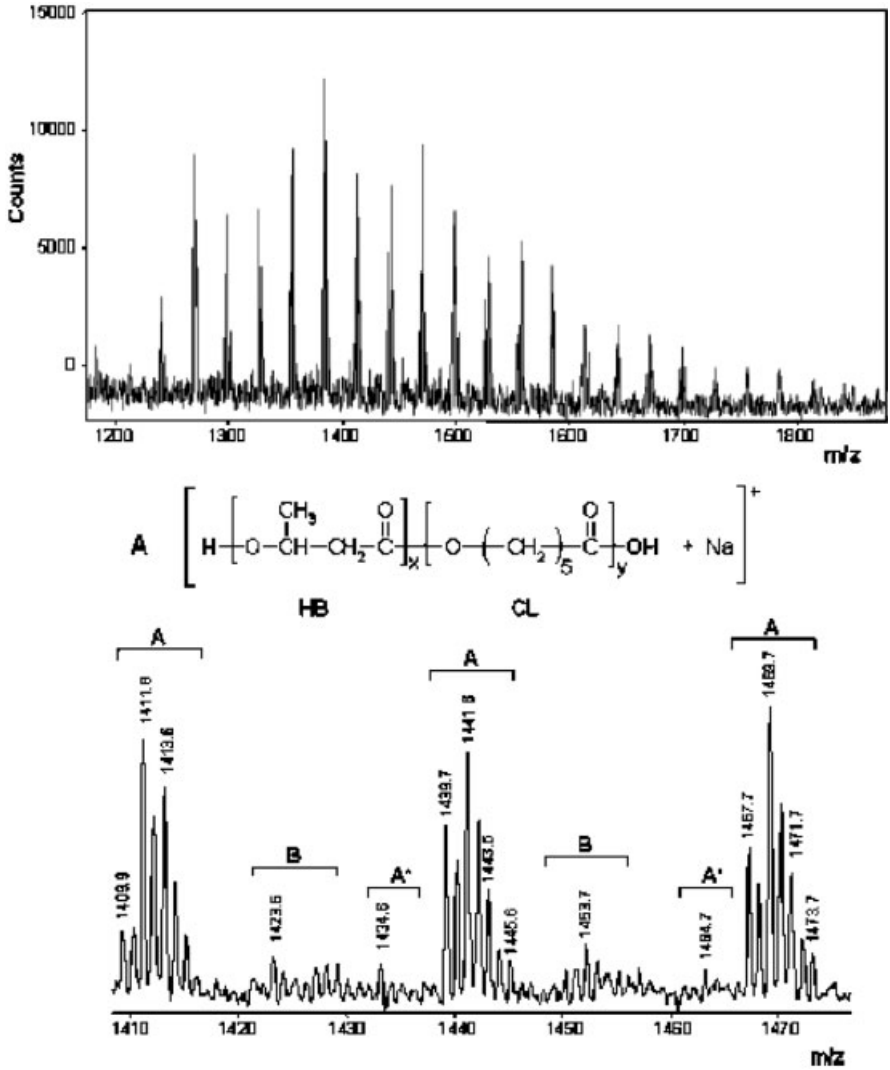


Figure 6.
the high-resolution MALDI-TOF mass spectrum of a relatively low MM fraction for P[(R,S)-3HB-co-CL].

been determined the optimal spinning parameters of the polymer: concentration of the spinning solutions: 30%; spinning temperature: 50 °C.

Characterisation of the Obtained Yarns

The obtained yarns were evaluated from the point of view of the physico-mechanical performances, the obtained results being presented in Table 1.

Experimental models of biomaterials with textile structures were made by technologies of knitting on rectangular knitting machines. The number of the needdles involved was $Na = 45 \times 2$, and the position of the looping clip adjusted adequately to 12,5. division.

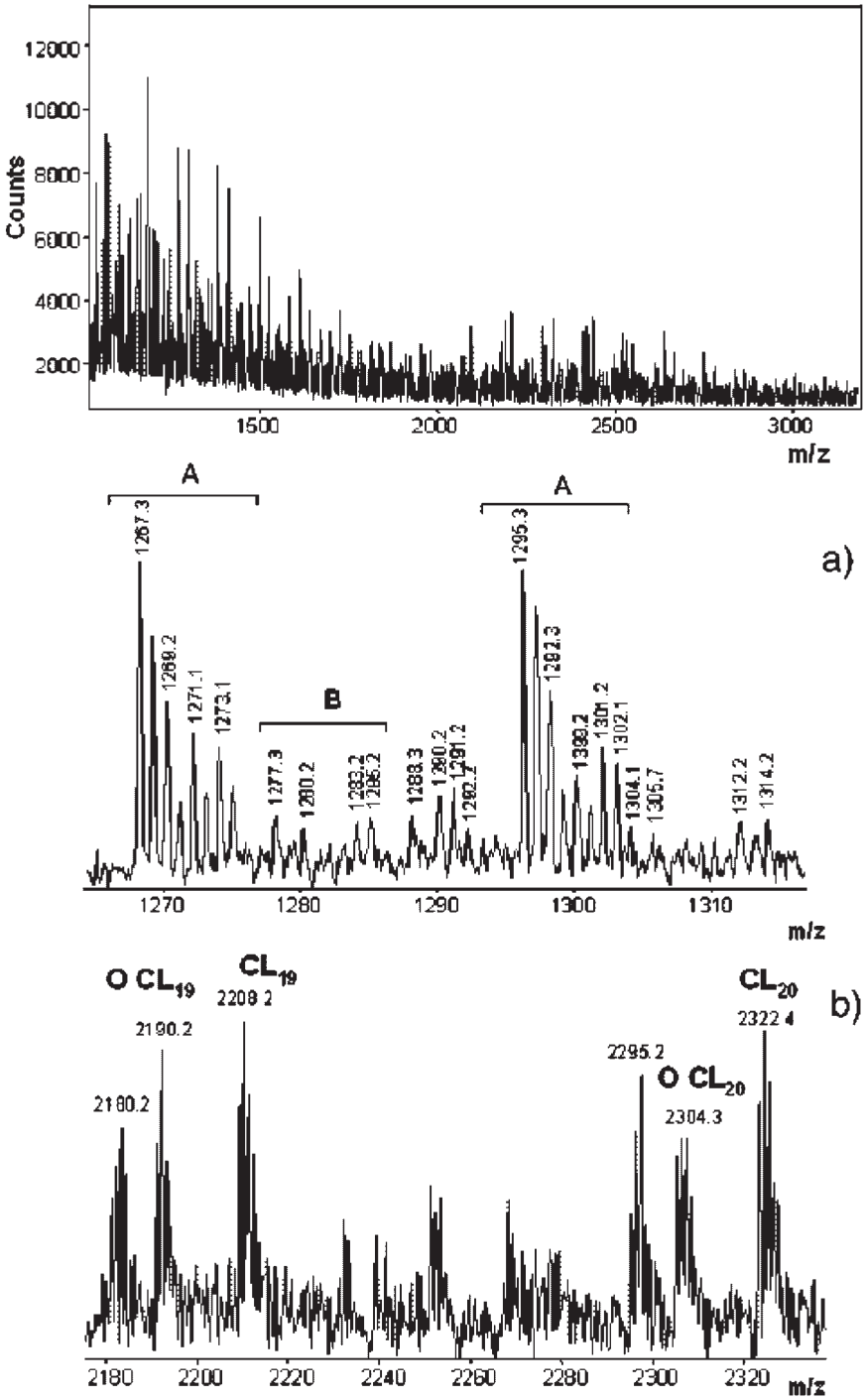


Figure 7.
The MALDI-TOF mass spectrum of unfractionated P[(R,S)-3HB-co-CL] (35:65) copolyester.

Table 1.
Physico-mechanical characteristics of the resorbable yarns.

Sample	Length Density dtex (den)	Resistance gf	Elongation %	Tenacity gf/dtex(gf/din)
1 NE	238,1 (214,3)	37	1,3	0,15 (0,17)
3 NE	228,3 (205,5)	43	1,6	0,19 (0,17)
3 E	235,8 (213,3)	93,2	2,2	0,39 (0,43)
4 E	206,2 (185,6)	105,9	3,4	0,51 (0,57)

Note: NE- nonstreched yarns; E-streched yarns.

Table 2.
Physical – chemical characteristics on watery extract.

Characteristics	Admission conditions	Obtained results
Appearance	Clear	Clear
Colour	Colourless	A little yellowish
Smell	None	None
Oxido-reducing substances,ml KMnO4; 0,01 N la 100 ml extract	Max.10	None
Content in $NH4^{+}$, g/100 ml extract	Max.0,0002	None
Content in Cl^{-}, g/100 ml extract	Max.0,00025	None
Content in $SO4^{2-}$, g/100 ml extract	Max.0,001	None
Content in heavy metals Pb^{2+}, g/100 ml extract	Max.0,0001	None
Content in Ca^{2+}, g/100 ml extract	Max.0,0003	None
Content in starch, dextrine	Max.0,3	None

The physical – chemical characteristics on watery extract of the obtained products were evaluated according to the methodology of the norms in progress for the category of medical implants, the obtained results being presented in Table 2.

Evaluation of Biocompatibility of the Biodegradable Copolymer

The biocompatibility studies had in view the tests of: toxical and hemolithical impurities, of acute toxicity in polar and nonpolar solvent, intradermal reactivity, sensitizing and cutaneous local tolerance. The tests were been carried out by the subcutaneous and intravenous introduction of the two extracts in Swiss mice and New Zeeland rabbits lots, the animals beeing kept under observation for 28 days. The obtained conclusions are: the lab animals had a good general state , the favorable weighing evolution, (they did not decrease in weigh); there weren't any toxical phenomena and mortality in any lot of treated animals; the polymeric film does not release in watery extract toxical and hemolithical impurities, it does not present any acute toxicity in polar or non polar solvent and intra dermal reactivity, it does not have sensitive potential and it is well tolerated and cutaned. Also, the copolymer had a good general tolerance, after oral administrating.

Conclusions

- The copolymeric material presents fiability and the fibre goes through a dry streching in relatively small ratio;
- The physical – mechanical determinations emphasized the streched and coagulated samples under the most gentle conditions presented the best mechanical – physical properties;
- The chemical – physical determinations the framing of all parameters within the admission limits stipulated by the norms for this products category;
- The carried out biological studies emphasized the biocompatibility of the copolymer PHBV/a-PHB, in for its using in the

manufacturing of the haemostatical dressings; the production is under the GMP rules.

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