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Critical properties of lactide-co-glycolide polymers for the use in microparticle preparation by the Aerosol Solvent Extraction System

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Abstract

The Aerosol Solvent Extraction System (ASES) process uses supercritical carbon dioxide for the production of microparticles. Since the critical temperature for this gas is at 304 K, polymers that are used in this process must fulfil certain requirements in crystallinity, and thermal behavior. This can be achieved by the use of blocked copolymers and thus the presence of semicrystalline microdomains in the polymers. However, changing the sequences of the comonomers dilactide and lactide often leads to polymers of low solubility due to long glycolide blocks. In this study, the critical properties of two blocked co-polymers were investigated, such as the blocked structure itself by ^{1}H-NMR and ^{13}C-NMR, the thermal behavior by differential scanning calorimetry (DSC), and the crystallinity by powder diffraction. The impact of these properties on microparticles formed by those polymers was also object of these studies. Additionally, two different model drugs, albumin and estriolm were embedded to investigate the impact of different polymer properties on drug content and release.

Keywords: Polylactide-co-glycolide; Microparticles; Supercritical fluids; NMR; Powder diffraction; Thermal analysis

1. Introduction

Polymers of lactic and glycolic acid are often used in pharmaceutics to formulate parenteral dosage forms with sustained release. Microparticles made from those polymers are easily suspendible in a suitable medium for subcutaneous or intramuscular injection (Tice and Gilley, 1985). There are several processes available to produce microparticles such as solvent evaporation, spray drying, or coacervation processes, leading to microparticles with often high residual solvent content or solvent removal in a second step by washing and drying the generated microparticles. The Aerosol Solvent Extraction System (ASES, Müller and Fischer, 1991) uses supercritical carbon dioxide to form microparticles and thus com-

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Table 1
Results of ^{1}H-NMR[a]

Polymer	I_{Lac}	I_{gly}	I_S	% Lac
PLGA 50/50	2.07	1	6.83	51.3
3 block	0.89	1	5.00	72.3

[a] I, relative signal intensitiy from NMR, the index Lac stands for lactoyl units, the index Gly for glycolyl units; L, block length, calculated according to Eqs. (1) and (2); I_S, signal intensity of the solvent (deuterated 1,1,1,3,3,3-hexafluoroisopropanol, HFIP).

bines formation and solvent removal in a one step procedure. The polymer/drug solution is sprayed into the supercritical gas phase where the polymer precipitates and incorporates the drug. The solvent is miscible with the gas and thus removed by the constant CO_2 stream. Since precipitation is an important mechanism for the microparticle formation in this process, suitable polymers must fulfil certain requirements in polymer size, crystallinity, and glass transition temperature.

In this study the physicochemical properties of two block copolymers were investigated. The substances were characterized by NMR spectroscopy, powder diffraction, and thermal analysis in order to find out their molar composition, the lengths of lactide and glycolide blocks, crystallinity and thermal behavior, because all these properties seem to have an influence on the ability of the polymers to form microparticles. Blocked copolymers proved to be very suitable for this process because they often are semicrystalline and thus have a better thermal behavior due to the presence of crystalline microdomains in the blocked blocks. The polymers were chosen because their different composition (i.e. molar and block composition) would make a clear difference in the investigations and thus prove one of the polymers to be suitable for the ASES.

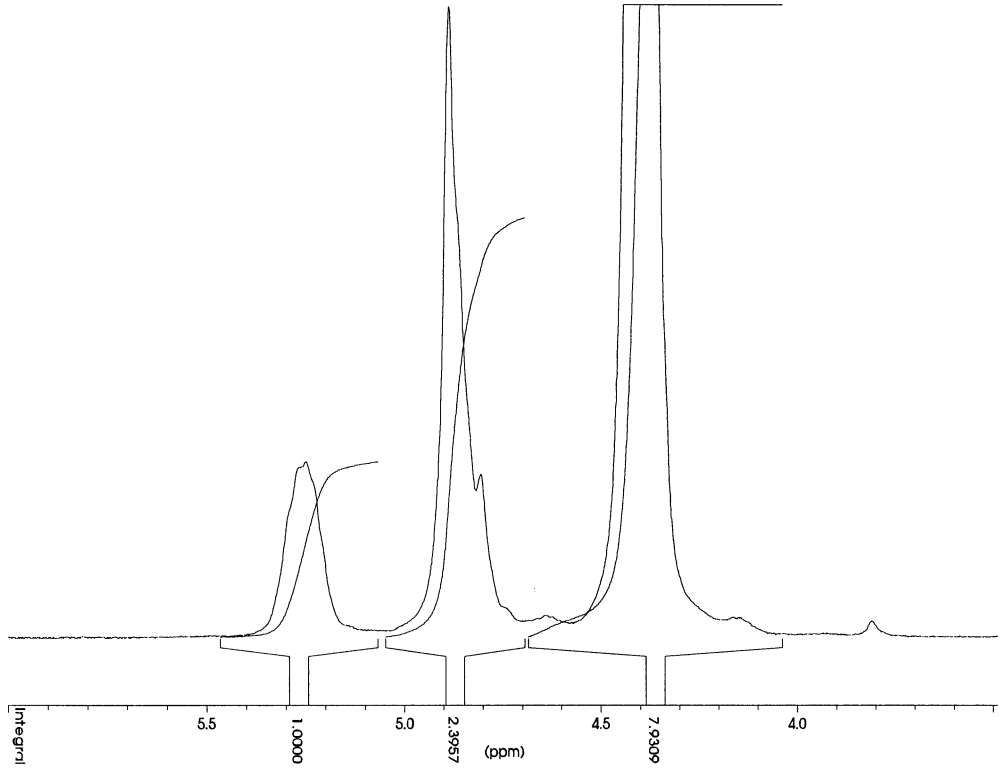

Fig. 1. ^{1}H-NMR spectrum of poly-D,L-lactide-co-glycolide (PLGA) 50/50.

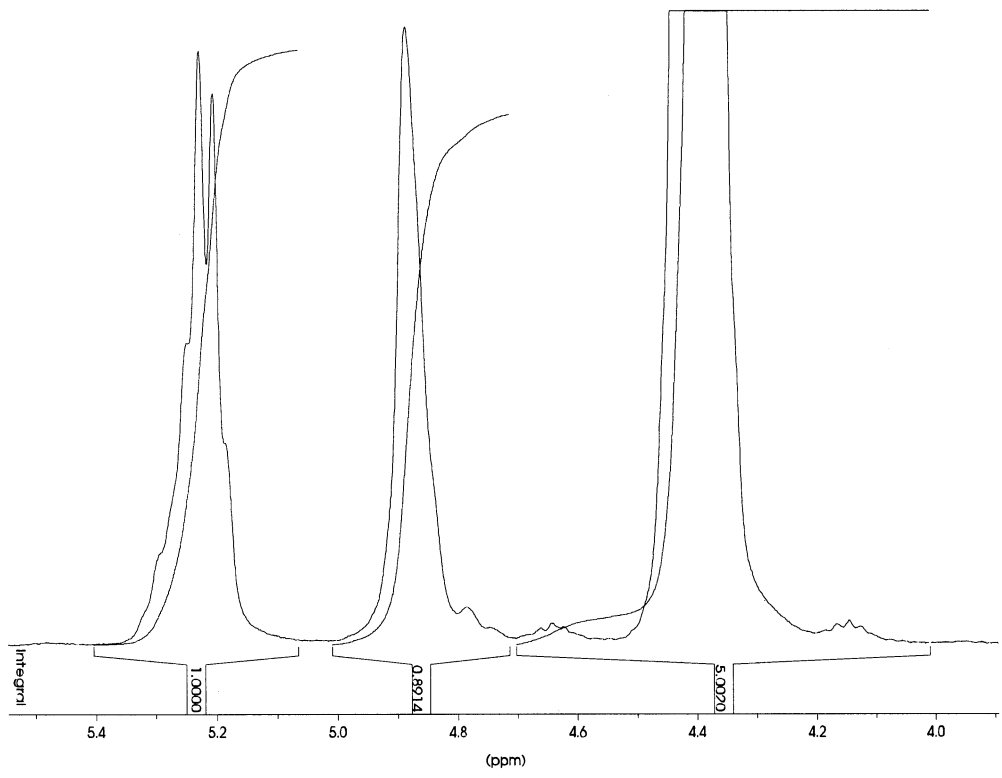

Fig. 2. ^{1}H-NMR spectrum of the 3 block polymer.

2. Material and methods

2.1. Polymers

A three-block-polymer (b-poly-L-lactide-co-D,L-lactide-co-glycolide 62.5:12.5:25) was purchased from the Fraunhofer Institute of Applied Polymer Chemistry (IAP-FhG), Teltow, Germany, synthesized by a transesterification method of Rafler and Müller (1996) and compared to a blocked poly-D,L-lactide-co-glycolide 50:50 (PLGA 50/50), that was obtained from the same manufacturer. The polymers were used as received and further named 'original polymer' to distinguish between polymers and microparticles.

2.2. Drugs and solvents

- Estriol (USP XXIII): Synopharm GmbH, Barsbüttel, Germany,
- Bovine serum albumin (BSA): fraction V, 96–99% Albumin, Sigma, St. Louis, MO,
- 2,2,2-Trifluoroethanol (TFE, analytical grade): Fluka Chemie AG, Buchs, CH was used as co-solvent for the polymers,
- Methylene chloride and Methanol (both analytical grade): Merck, Darmstadt, Germany.

2.3. NMR spectroscopy

All measurements were carried out in a Bruker ARX 300 NMR spectrometer (Bruker, Rheinstetten, Germany) at a temperature of 300 K. The polymers were dissolved in deuterated 1,1,1,3,3,3-hexafluoroisopropanol (HFIP-d, degree of deuteration 98.5%, purchased from Sigma, Deisenhofen, Germany) to guarantee the solubility of all specimens. The concentration of the polymers in the solution was 10%. The ^{1}H-NMR spectra were registered at a frequency of 300.13 MHz. The chemical shift is given in relation to the internal

standard tetramethylsilan. The ^{13}C-NMR spectra were recorded by inverse gated decoupling to reduce the nuclear Overhauser enhancement (NOE). The chemical shift was also given in relation to the internal standard TMS. The recording frequency was 75.47 MHz, sweeping over 16 700 Hz. Two thousand records were accumulated. The time between two pulses for complete relaxation of the ^{13}C-nuclei was determined with 5 s.

2.4. Thermal analysis

Thermal analysis of both, original polymers and the drug-free microparticles was carried out using a Perkin Elmer DSC 7 System (Perkin Elmer, Norwalk, CT). The temperature was scanned from −5 to 250°C and backward at a heating/cooling rate of 10°C.

2.5. Powder diffraction

Powder diffraction was carried out in a Siemens D500 apparatus (Siemens, Germany) with a stationary anode at a wave length of 1.54 Å.

2.6. Scanning electron microscopy (SEM)

Microparticle pictures were taken using a Philips XL 20 scanning electron microscope (Philips, Kassel, Germany). The samples were fixed on a carbon film and sputtered with gold in an Argon atmosphere at a sputter current of 50 mA for 180 s using a SCD 005 Sputter coater (BAL-TEC, Balzers, Liechtenstein). The high tension in the SEM was set at 10–20 kV, so that the microparticles did not soften under the thermal burden and form artefacts.

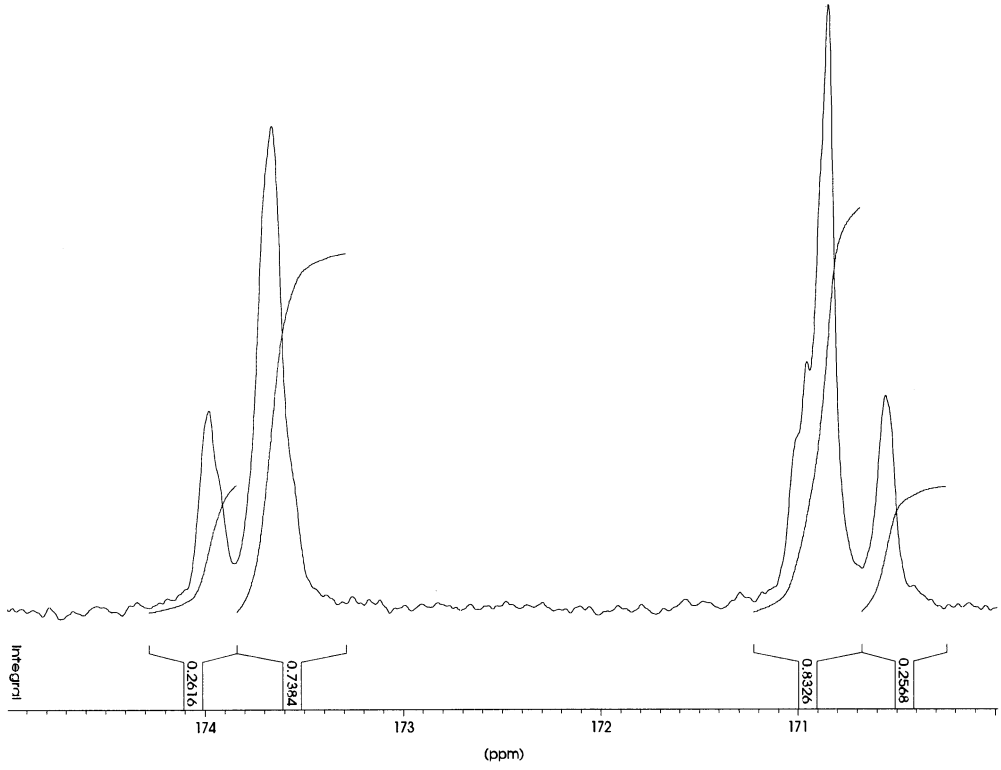

Fig. 3. ^{13}C-NMR spectrum of poly-D,L-lactide-co-glycolide (PLGA) 50/50.

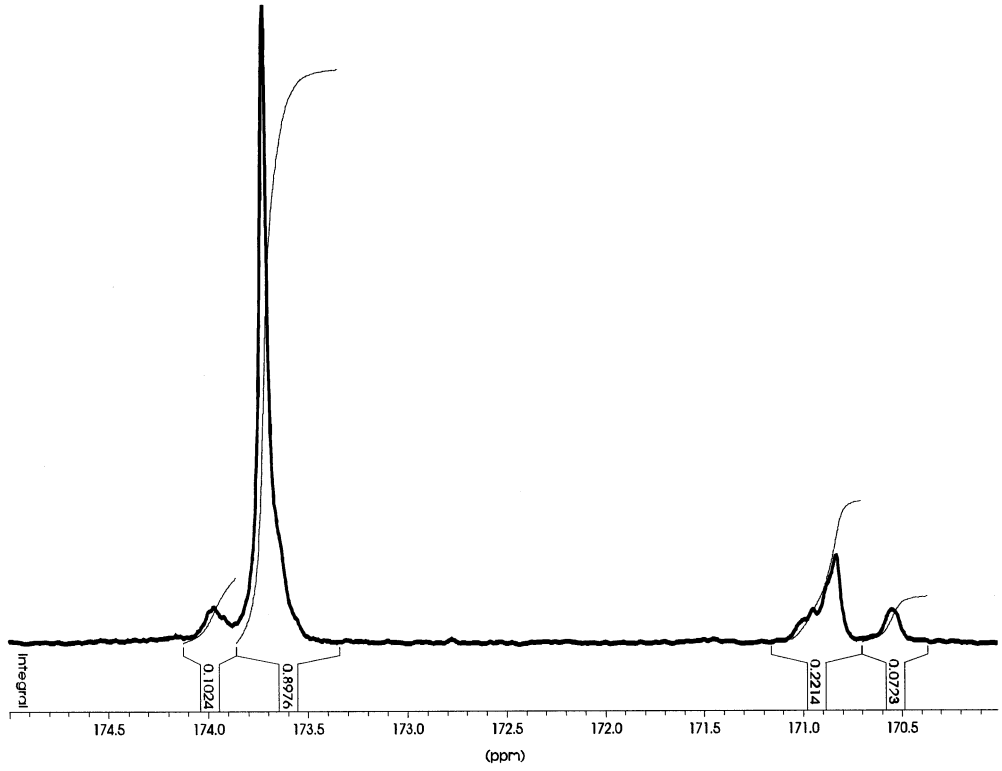

Fig. 4. ^{13}C-NMR spectrum of the 3 block polymer.

Table 2
Results of ^{13}C-NMR-peak intensities and calculated values[a]

Polymer	$I_{lac-Lac}$	$I_{Lac-Gly}$	$I_{Gly-Gly}$	$I_{Gly-Lac}$	ΣI	% Lac	L_{Lac}	L_{Gly}
PLGA 50/50	0.8	0.2	0.75	0.19	1.94	51.6	3.8	4.2
3 block	0.90	0.10	0.22	0.07	1.29	77.3	9.8	4.1

[a] I, relative signal intensity from NMR, the index Lac stands for lactoyl units, the index Gly for glycolyl units; L, block length, calculated according to Eqs. (1) and (2); I_S, signal intensity of the solvent (deuterated 1,1,1,3,3,3-hexafluoroisopropanol, HFIP).

2.7. Microparticle production

For the drug free batches 3.0 g of the polymers (2.85 g for the batches with drug) were dissolved in 10 g TFE and 10 g CH_2Cl_2. After complete dissolution of the polymers this concentrated solution was diluted with CH_2Cl_2 to a concentration of 3% by mass. When a drug was incorporated, 0.15 g of the drug (estriol or BSA) were dissolved in 20 ml methanol. This solution and the solution of the polymers were mixed and thinned with CH_2Cl_2 as described above.

The ASES apparatus was kept at a pressure of 10 MPa at a temperature of 307 K during the production period. The CO_2 circulated at a pump rate of 11 kg/h. The drying process was carried out over 3 h. The solution was sprayed into the supercritical gas phase by an HPLC pump (Model Economy, Techlab, Eckerode, Germany) at a pump rate of 6.00 ml/min. The circulation of the carbon dioxide was started 30 min after the polymer solution had been sprayed in.

2.8. Particle size

The particle size was determined using a laser diffractometer (Sympatec HELOS, Clausthal, Germany). The Fourier lens was focussed at 100 mm. Particles were suspended in a 0.1% solution of Pluronic F 68 in water. The particle size was determined before and after 90 s ultrasonication to see the agglomeration tendency.

2.9. Drug content

2.9.1. Estriol

The content of estriol was determined, after 24 h hydrolyzation of the particles in 0.25 N NaOH, using a UV-Spectrophotometer (Uvikon 810, Kontron, Zurich, CH) at $\lambda = 296$ nm. The measurements were carried out in the hydrolyzed solution.

2.9.2. BSA

The content of BSA was determined, after 24 h hydrolyzation of the microparticles in 0.25 NaOH, using a UV-Spectrophotometer (Uvikon 810, Kontron, Zurich, CH) at $\lambda = 280$ nm. The measurements were carried out in the hydrolyzed solution.

2.10. Drug release

The particles were suspended in a phosphate buffer (Sörensen) at pH 7.4 containing 0.1% Pluronic F 68 and 2% methyl-β-CD over 24 h at a temperature of 37°C. The residual drug content in the particles was determined by filtering the suspension, hydrolyzing the collected microparticles and determining the content as described above.

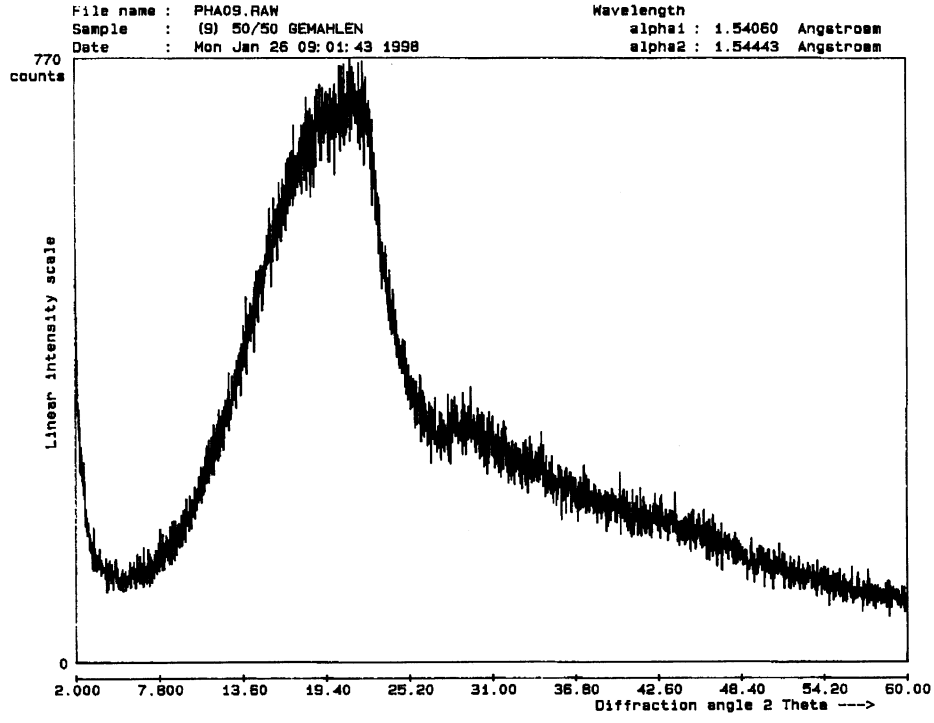

Fig. 5. Powder diffraction pattern of poly-D,L-lactide-co-glycolide (PLGA) 50/50.

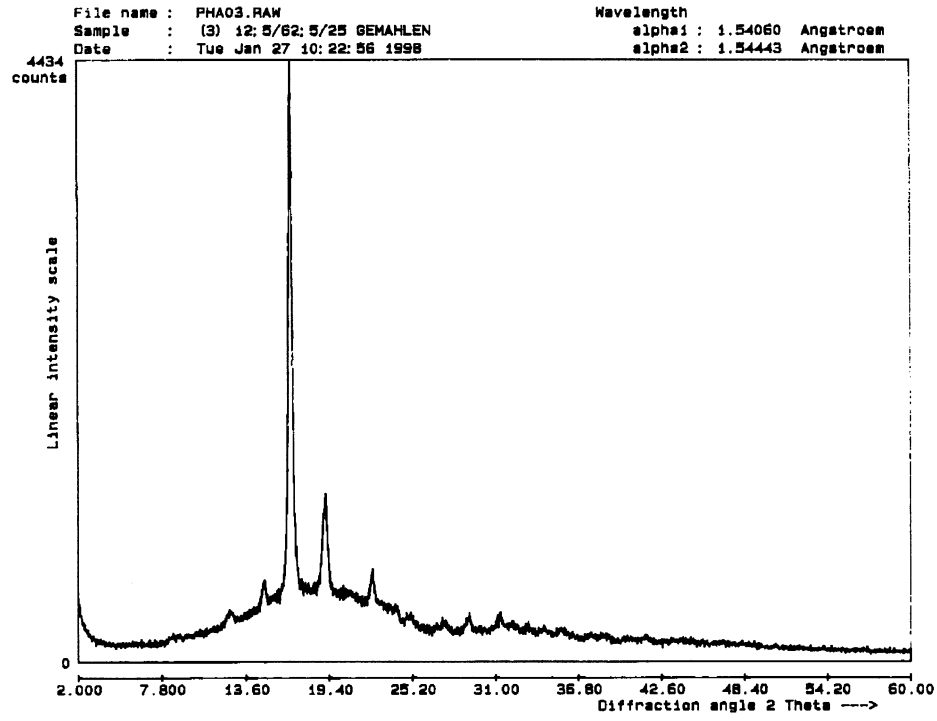

Fig. 6. Powder diffraction pattern of the 3 block polymer.

3. Results and discussion

3.1. NMR spectroscopy

The proton NMR spectroscopy was applied to verify the manufacturer's declarations about the molar ratio of the polymers that were derived from the amounts added to the synthesis. The results obtained from NMR analysis correspond very well with these declarations. The ^{1}H-NMR results are given in Table 1, spectra are presented in Figs. 1 and 2. According to a method published by Kricheldorf and Kreiser (1987), ^{13}C-NMR was applied to obtain information regarding the molar fractions as well as on the length of lactide and glycolide blocks. Due to γ-effects (effects caused by a substituent in γ-position) the carbonyl signal splits in to pairs of signals standing for lactide–lactide bonds (Lac–Lac) and lactide–glycolide bonds (Lac–Gly) on one hand and glycolide–glycolide bonds (Gly–Gly) and glycolide–lactide bonds (Gly–Lac) on the other hand (Braun et al., 1996). The Figs. 3 and 4 and show spectra of the investigated polymers. It is thus possible to calculate the length of lactide and glycolide blocks (block lengths, L) in the polymer from the relative signal intensities (I) according to the following equations:

$$L_{\mathrm{Lac}} = \frac{I_{\mathrm{Lac-Lac}}}{\mathrm{I}_{\mathrm{Lac-Gly}}} + 1 \quad (1)$$

$$L_{\mathrm{Gly}} = \frac{I_{\mathrm{Gly-Gly}}}{I_{\mathrm{Gly-Lac}}} + 1 \quad (2)$$

The spectra as well as the calculations showed that there are longer blocks of each lactide and glycolide in both polymers. Due to the different molar compositions, the ratio of the blocks is higher for the three-block-polymer than for the PLGA 50/50 (Table 2). Both polymers have a glycolide block length of more than 4 (Table 2). Long glycolide sequences clearly decrease the solubility of the polymers in methylene chloride as preliminary studies (Engwicht et al., 1998) have shown. The different optical properties (L-lactide

or D,L-lactide) of the polymers are not detectable in the spectra.

3.2. Powder diffraction analysis

After it had been proved that both polymers have a blocked structure it was interesting to see if this had consequences on the crystallinity of the polymer. Harland and Peppas (1993) reported that blocked co-polymers are able to form microdomains which cause a partial crystallinity in the substance. The diffraction patterns (Figs. 5 and 6) show that the PLGA 50/50 is completely amorphous in spite of its blocked structure. The three-block-polymer on the other side is partially crystalline due to the sharp peaks sitting on a halo reaching from 10 to 30° 2θ. This difference can be explained by the long sequence of amorphous glycolide in the PLGA 50/50 that still determines the rate of crystallinity for the whole polymer. In addition, the lactide part consists of D,L-lactide, which is amorphous, too. The high amount of crystalline poly-L-lactide (62.5%) on one hand and the fact that all three components in the polymer are separated in blocks on the other hand improve the crystallinity of the polymer.

3.3. Thermal analysis

The results obtained from the powder diffractometry were confirmed when the polymers were investigated by differential scanning calorimetry (DSC). The amorphous PLGA 50/50 shows several peaks that may prove the blocked character of the polymer (Fig. 7). Li et al. (1996) observed such a phenomenon in the DSC curve of a blocked copolymer from L,L-dilactide and polyethyleneglycol and could interpret the different peaks as glass transition of the lactide or melting of the PEG. The DSC curve of the three-block-polymer shows a distinct peak in the high temperature range of the curve (Fig. 8). This peak was seen as a melting point of the L-lactide part in the polymer. The thermal behavior in a tempera-

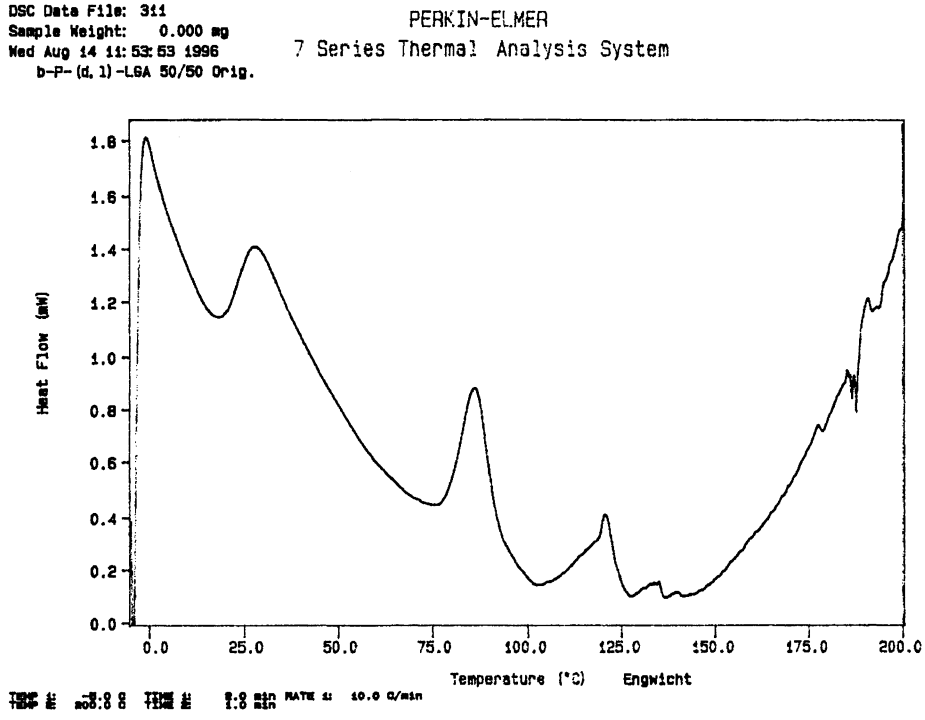

Fig. 7. Differential scanning calorimetry (DSC) curve of poly-D,L-lactide-co-glycolide (PLGA) 50/50.

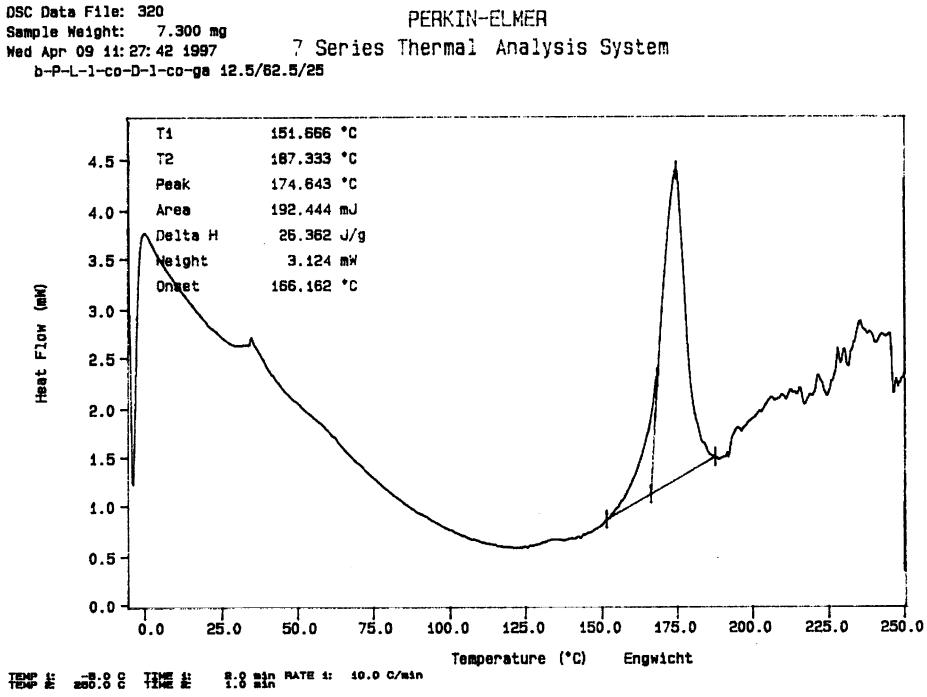

Fig. 8. Differential scanning calorimetry (DSC) curve of the 3 block polymer.

ture range from 30 to 50°C is interesting for the applicability of the polymers, because the ASES process is commonly run at temperatures between 33 and 40°C. The PLGA 50/50 shows events in this region and the precipitated particles may thus soften during the process and much agglomeration may occur.

3.4. Particle morphology

The SE-micrographs (Figs. 9 and 10) indicated, that products of different morphology were obtained. While the PLGA 50/50 particles are quite small, of irregular form, and highly agglomerated, the particles of the three-block-polymer are bigger, of a round shape, and appear more individual. Moreover, these particles are porous, which was not observed with the microparticles of the PLGA 50/50.

3.5. Particle size, drug content and release

The results of the particle size measurements for all batches are shown in Figs. 11 and 12. Fig. 11 presents the median $d_{50\%}$ of the particle size before and after 90 s ultrasonication of the sam ple, Fig. 12 shows the agglomeration index, i.e.

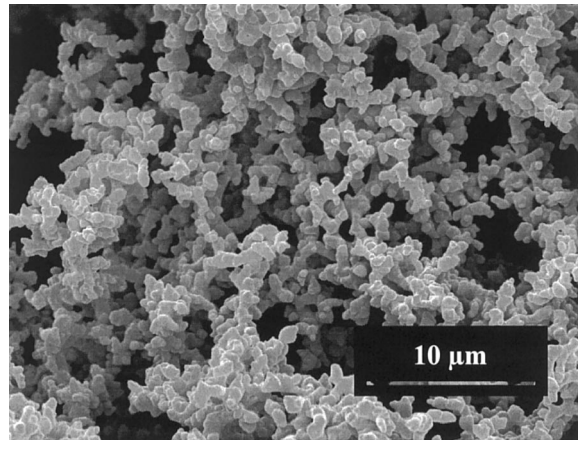

Fig. 9. Scanning electron microscopy (SEM) picture of particles made from poly-D,L-lactide-co-glycolide (PLGA) 50/50.

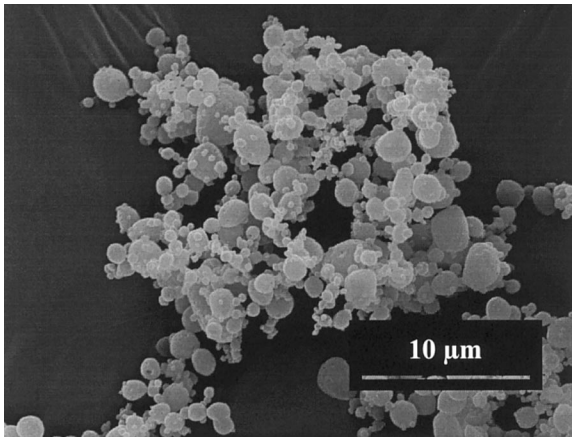

Fig. 10. Scanning electron microscopy (SEM) picture of particles made from the 3 block polymer.

the ratio between the medians before and after ultrasonication, and the span between the quantile $d_{90\%} - d_{10\%}$ in µm. The impression obtained from the SEM is confirmed by the LD measurements: although the PLGA 50/50 particles appeared much smaller, the size of them is distinctly bigger due to the presence of agglomerates. Consequently the agglomeration index for these particles is quite high. These agglomerates cannot completely be destroyed by ultrasonication of the sample over 90 s. The span $d_{90\%} - d_{10\%}$ is still very large, indicating the presence of big agglomerates. The particle size for the product of the three-block-polymer does not change so much before and after ultrasonication, indicated by the low agglomeration index. Also the span $d_{90\%} - d_{10\%}$ for these particles is very low since the agglomerates are neither big nor numerous. The explanation for this is given by the different thermal properties of the polymers. The PLGA 50/50 softens indeed as presumed in the discussion of the DSC results. The particles rest in the warm carbon dioxide atmosphere for three hours at a temperature close to their glass transition temperature. Moreover, in the first 30–60 min there was a high solvent concentration in the gas phase, which may additionally depress the low T_g of the co-polymer. Furthermore, carbon dioxide is also partially soluble in the polymer which may also lead to an even lower T_g than the experimentally obtained one. So the particles float together and form firm agglomerates that cannot be easily redispersed even by ultrasound. The presence of

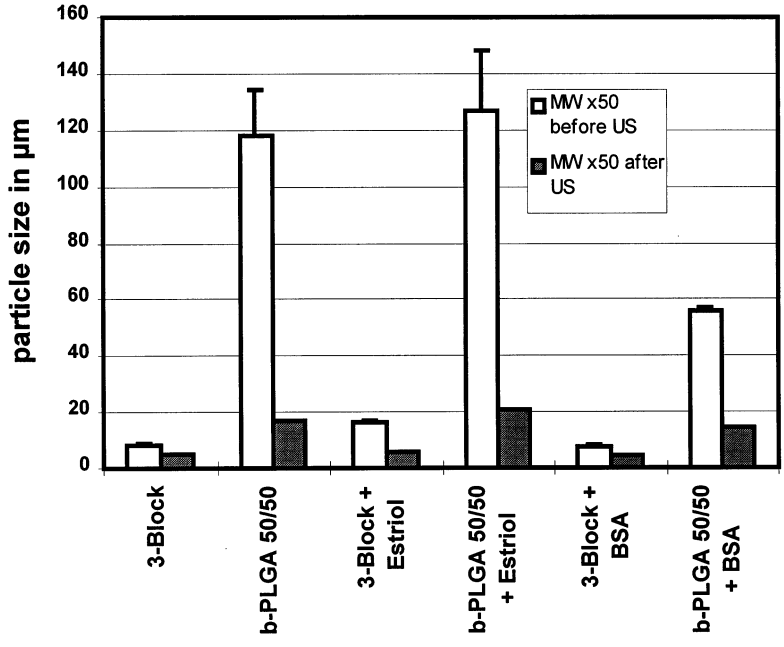

Fig. 11. Particle size ($d_{50\%}$) of microparticle batches before and after ultrasound dispersion.

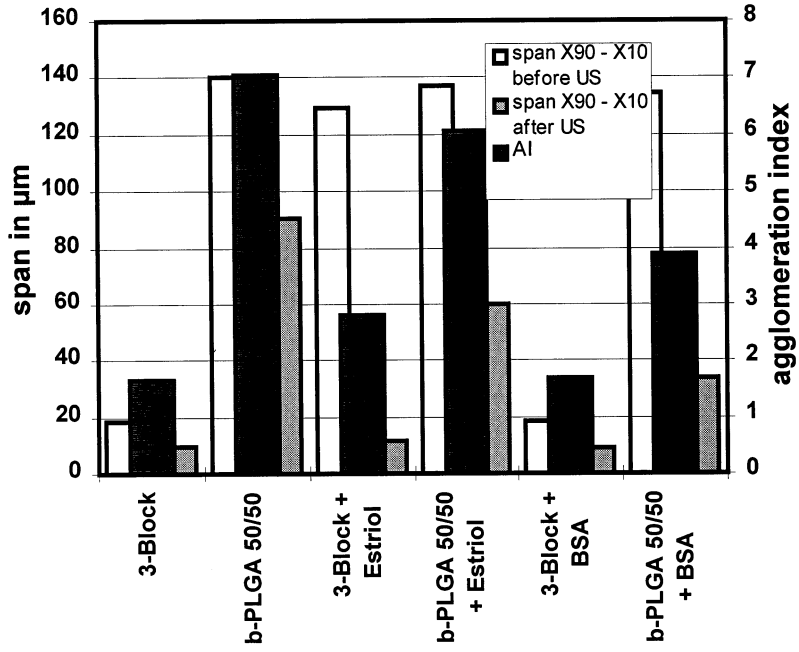

Fig. 12. Span $d_{90\%} - d_{10\%}$ of the microparticle batches and agglomeration index (AI).

Table 3
Drug content in microparticles and residual drug content after 24 h[a]

	Drug content in microparticles (%)		Residual amount after 24 h hydrolyzation (%)	
	PLGA 50/50	3 block	PLGA 50/50	3 block
Estriol	110.01	103.21	56.71	55.68
BSA	114.55	105.6	48.19	52.98

[a] I, relative signal intensitiy from NMR, the index Lac stands for lactoyl units, the index Gly for glycolyl units; L, block length, calculated according to Eqs. (1) and (2); I_S, signal intensity of the solvent (deuterated 1,1,1,3,3,3-hexafluoroisopropanol, HFIP).

Estriol does not influence the particle size, the agglomeration index or the span of the three-block-polymer but makes these values worse for the PLGA 50/50. The cause may be that most of both drugs is located at the surface of microparticles. The hydrophilic BSA thus improves the wettability of the hydrophobic polymer surface especially of the PLGA 50/50, while the hydrophobic estriol which was micronized and highly agglomerated itself has a more negative influence on this parameter. The speculation about the location of the drugs with respect to the microparticles is confirmed by the results given from the drug content determinations (Table 3) and the release studies. The high drug contents (above 100%) in the microparticles may be explained by a partial extraction of lower molecular weight fractions of the polymers during the process. Both drugs are not soluble in CO_2 at the conditions the process was run because they carry too many polar groups or are too big, respectively, and all the drug amount weighed was refound by the drug content determinations. After 24 h in the drug release tests only 50–60% of residual drug was found in the microparticulate matrix (Table 3). So there is a high burst rate within this time in the microparticles caused by the portion of drug at the surface or right below it.

4. Conclusions

In this study, two blocked lactide-co-glycolide polymers, synthesizised previously, were compared. Moreover, microparticles were produced by means of the ASES process to see if differences in the polymer properties lead to different product characteristics. Shape and morphology of microparticles were characterized by SEM, particle size was determined by laser diffraction. Apart from blank microparticles, batches containing either estriol or albumin on the other hand were produced to characterize embedding and release of these drugs. The blocked structure of the investigated polymers was proved by NMR analysis. However, in a polymer where lactide and glycolide are present in a ratio 50:50, the blocked structure is not sufficient to generate crystalline structures, especially when the lactide sequence consists of D,L-lactide. Better results in regard to crystallinity were shown by a three-block-polymer composed of L-lactide/D,L-lactide and glycolide 62.5%/12.5%/25%. This was proved directly by powder diffraction spectroscopy. DSC measurements showed a clear difference in the thermal behavior for both polymers. While the three-block polymer showed a melting point at ca. 170°C, only a glass transition phenomenon was observed for the other substance. Consequently the microparticles produced from both polymers differed substantially in shape, size, and their agglomeration tendency. Due to the low glass transition temperature, the particles made from PLGA 50/50 soften during drying in the high pressure column and form big agglomerates. These can only partially be redispersed by 90 s ultrasonication treatment of the sample in the laser diffractometer. The differences in the physico-chemical properties of the polymers, however, do not influence the embedding or the release of the drugs that were used in this study. The drugs are primarily located on the particles' surface, which was verified by release tests, showing a high burst rate for both specimens. It can thus be concluded that the used three-block polymer is more applicable for the production of microparticles than the PLGA 50/50 because of its higher crystallinity and its better thermal behavior. The microparticles are smaller and have a lower agglomeration tendency. Hence this polymer is a promising candidate for further developments.

References

Braun, S., Kalinowski, H.-O., Berger, S., 1996. 100 and More Basic NMR Experiments—A Practical Course. Verlag Chemie, Weinheim, p. 234f.

Engwicht, A., Girreser, U., Müller, B.W., 1998. Characterization of co-polymers of lactic and glycolic acid by means of NMR spectroscopy, in press.

Harland, R.S., Peppas, N.A., 1993. Drug transport in and release from controlled delivery devices of hydrophilic/hydrophobic block and graft polymers. J. Pharm. Biopharm. 39, 229–233.

Kricheldorf, H.R., Kreiser, I., 1987. Kationic copolymerization of glycolide with L,L-dilactide. Makromol. Chem. 188, 1861–1873.

Li, S., Rashkov, I., Espartero, J.L., Monolova, N., Vert, M., 1996. Synthesis, charactrization, and hydrolytic degradation of PLA/PEO/PLA triblock copolymers with long poly(L-lactic acid) blocks. Macromolecules 29, 57–62.

Müller, B.W., Fischer, W., 1991. Method and apparatus for the manufacture of a product having a substance embedded in a carrier. US patent No. 5,043,280.

Rafler, G, Müller, B.W., 1996. Verfahren zur Herstellung biologisch abbaubarer Polyester, EP-PS 0 573 024.

Tice, T.R., Gilley, R.M., 1985. Preparation of injectable controlled release microcapsules by a a solvent evaporation process. J. Control. Rel. 2, 343–352.