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Poly(L-lactide) microspheres with controlled crystallinity

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Abstract

Biodegradable polyester microspheres were synthesized directly from L-lactide by ring-opening dispersion polymerization. Diameter distributions of these particles were narrow ($D_w/D_n < 1.27$), molecular weight of polymer constituting microspheres ranged from 7 to 270 kDa, and its molecular weight polydispersity was low ($M_w/M_n < 1.2$). Degree of crystallinity of poly(L-lactide) in microspheres was controlled in the range from 0 to 60%, by the appropriate thermal treatment of suspensions of microspheres. This procedure did not affect diameters of microspheres, their colloidal stability, and molecular weight of poly(L-lactide) in polymer particles. It has been established that the determination of the degree of crystallinity of poly(L-lactide) in microspheres by ^{13}C CP-MAS NMR yielded results more reproducible than determined by DSC.

Keywords: Polylactide microspheres; Crystallinity; Microparticles

1. Introduction

Microspheres and other dispersed systems made of biodegradable polyesters, in particular poly(ϵ-caprolactone), poly(lactic acid), and/or poly(lactic acid-*co*-glycolic acid), are used as carriers of anticancer drugs, biologically active amines, contraceptive, anabolic, and antiinflamatory steroids, antibiotics, antimicrobial agents, polypeptides (e.g. vaccines) [1–4]. Microspheres are usually obtained from earlier synthesized polymers by the precipitation–evaporation related methods [5–9]. Diameters of microspheres produced in this way can be controlled in range from 5 to 400 μm, but the polydispersity of diameters is very high (coefficient of variation CV ≈ 30%). There are some evidences that degradation rate of the poly(lactic acid) polymer matrix depends on the size and geometry of the polymer sample [10]. It is not clear to which extent this observation does apply also to micron size objects, however, control of diameter distribution of polymer microspheres used as drug carriers is considered to be of primary importance.

Recently, we developed a method of synthesis of biodegradable polyester microspheres with controlled diameters (from 2.0 to 6.4 μm) and with narrow diameter distribution, directly by a ring opening dispersion polymerization of lactides or lactones [11–14]. Microspheres with such diameters should be able to pass the capillary blood vessels and possibly also to cross the M cells layer of the digestive tract. Surface properties of these microspheres could be modified by the adsorption of proteins (e.g. human serum albumin (HSA) and human gamma globulins (γG)) [11].

Suitability of biodegradable microspheres for application as drug carriers depends on the rate of drug release, which is affected by chemical composition, molecular weight, and also by the degree of crystallinity of polymer matrix [15]. Moreover, crystallinity of polymers introduced into organisms affects their immunological response [16,17].

There were reports on attempts to produce microspheres with controlled crystallinity from polylactides [15]. The main goal of this work was to develop a method suitable for preparation, in the combined processes of the ring-opening polymerization of L-lactide and after synthesis treatment, the uniform PLLA microspheres with controlled degree of crystallinity. We did hope that this could be achieved by annealing the synthesized microspheres in the appropriate medium and at the required temperature.

2. Experimental procedures

2.1. Synthesis

Detailed description of purification of reagents and of the synthesis of microspheres was given in our earlier publications [11–13]. Here we recall briefly only the most important data. In a typical synthesis 4.0 g of L-lactide and 0.16 g of poly(dodecyl acrylate-*g*-ϵ-caprolactone) (surface active

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Table 1
Initial initiator concentrations, molecular weights of poly(L-lactide) in particles, particle diameters, and diameter polydispersity factors

No	$[I]_0 \times 10^3$ (mol l^{-1})	M_n	M_w/M_n	D_n (μm)	D_w/D_n
1	4.03	7000	1.06	2.49	1.17
2	1.99	8600	1.06	4.07	1.23
3	4.72	12,200	1.08	2.58	1.07
4	5.42	16,400	1.11	3.45	1.05
5	5.78	34,200	1.18	2.28	1.10
6	8.02	42	1.18	2.76	1.06
7	4.10	57,700	1.13	2.43	1.12
8	5.24	127	1.16	1.76	1.27
9	2.83	150	1.20	1.92	1.23
10	3.98	270,000	1.13	1.71	1.08

agent) were dissolved in a boiled mixture of 70 ml of *n*-heptane and 20 ml of 1,4-dioxane. Then, a solution of 0.22 g of tin(II) 2-ethylhexanoate in 10 ml of heptane was added, and polymerization was carried out with stirring (60 rpm) at 95°C. Preparation of reaction mixture and polymerization were made in dry argon atmosphere. Polymerization was stopped after 2 h and then the reaction mixture was cooled down at room temperature for 90 min. Thereafter, 100 ml of cold heptane (100 ml) was added to this mixture and precipitated crystals of the remaining monomer were removed by fractional sedimentation. The yield of microspheres was 65%.

2.2. Analyses

Molecular weight and molecular weights distributions of poly(L-lactide)s constituting microspheres were determined using a Knauer vapor pressure osmometer (tonometer), a Knauer membrane osmometer, and a gel permeation chromatography system combining a LKB 2150 pump, TSK-Gel G6000HR and G3000HR columns (TOSOHAAS), Dawn F MALS detector (Wyatt), and Optilab 903 Interferometric Refractometer (Wyatt). Dichloromethane was used as an eluent with a flow rate 0.8 ml min^{-1}. The data collected by Dawn F MALS detector were analyzed using Aurora v. 2.1 software. Diameters of microspheres were determined from microphotographs registered using a JEOL 35C scanning electron microscope. SEM pictures were analyzed using a Multiscan v. 6.08 software (Computer Scanning System, Warsaw, Poland). DSC curves were registered and analyzed using a DuPont 2000 Thermal Analysis System. In these measurements the heating rate was constant and equal to 10°C min^{-1} for each sample. Indium standard was used for calibration.

^{13}C CP-MAS NMR spectra were acquired using a Bruker MSL 300 MHz spectrophotometer (operating at 75.468 MHz for ^{13}C) equipped with cross polarization, magic-angle spinning (MAS) probe and at high-power decoupling conditions. Samples were placed in 4 mm zirconium rotor (volume 0.08 cm^3) and rotated with speed 8.2–8.4 kHz. All spectra (8192 data points) were registered using a spectral width of 25 kHz, contact time of 4 ms, and 6 s interval between acquisitions. Typically, 1024 spectra were averaged for each sample. The carbonyl region of the ^{13}C CP-MAS NMR spectra was deconvoluted into components arriving from a crystalline and an amorphous fraction by using the PeakFit program (Jandel Scientific Software, USA).

2.3. Annealing of samples

For annealing the poly(L-lactide) microspheres suspended in heptane were heated at constant temperature. Aliquots of suspension withdrawn at chosen time intervals were injected into the five-fold excess of cold heptane (−78°C). With purpose to obtain fully amorphous microspheres the following procedure has been used. First, microspheres were transferred from heptane to dodecane. Then, this suspension was heated for 20 min at a temperature of 175°C (reflux of dodecane) under dry argon atmosphere and finally, was purged into the five-fold excess of cold heptane. During melting and annealing suspensions were stirred to prevent sedimentation of particles. After annealing microspheres were dried for 24 h on air at room temperature, and eventually for 8 h in vacuum.

3. Results and discussion

3.1. Diameters, diameter distributions of microspheres. Molecular weights and molecular weight distributions of poly(L-lactide) in microspheres

Values of diameters and diameter distributions of microspheres synthesized in various syntheses are listed in Table 1. In Table 1 the values of M_n and M_w/M_n of poly(L-lactide)s constituting microspheres are also given. It is worth noting that while the molecular weights of poly(L-lactide) covered the broad range from 7000 to 270 000 the molecular weight distributions were narrow ($M_w/M_n \leq 1.2$). Number average diameters of microspheres ranged from 1.7 to 4.1 μm. Values of diameter polydispersity factor ($D_w/D_n \leq 1.27$) indicated that microspheres used in subsequent experiments were close to monodisperse.

3.2. Annealing

An effect of annealing of dry poly(L-lactide) microspheres at temperatures above T_g onto their crystallinity was investigated by Cuadrado et al. [18]. However, it is very well known that at these conditions the annealing of particulate materials results in coalescence and deformation of particles, which eventually forms an uniform film. On the other hand, at temperatures below T_g, the annealing does not lead to any changes of morphology of microspheres. Some authors reported on poly(L-lactide) microspheres loaded with bioactive compounds which, even when stored below T_g of poly(L-lactide), did change their morphology [19].

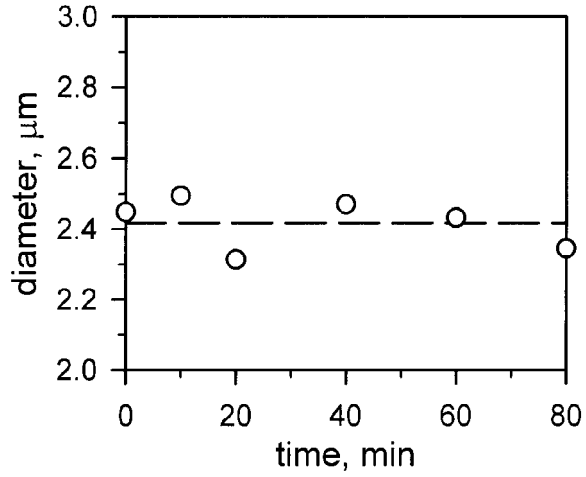

Fig. 1. Dependence of the number averaged diameter D_n of poly(L-lactide) ($M_n = 7000$) microspheres on the time of annealing at 80°C. Dashed line corresponds to averaged D_n.

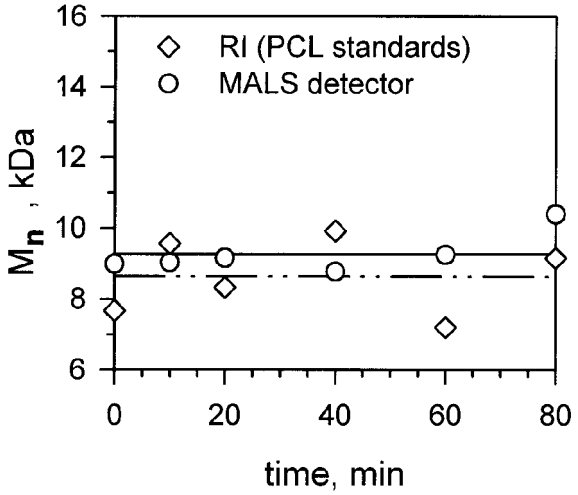

Fig. 2. Dependence of the number average molecular weight (M_n) of poly(L-lactide) on time of annealing at 80°C. Dashed line represents averaged value of M_n.

These changes were related to plasticizing effect of bioactive additives [20].

In our studies we wanted to find a method allowing to change in the controlled way the crystallinity of poly(L-lactide) microspheres without affecting their diameters. The first attempt to control the crystallinity of microspheres was based on various procedures of cooling the polymerizing mixture. We suspected that slow cooling of the mixture from 95°C to room temperature with rate 1°C min^{-1} will yield a more crystalline polymer than rapid cooling bellow T_g (−20°C). Unfortunately, both the procedures yielded microspheres with degree of crystallinity differing only a little (for poly(L-lactide) with molecular weight 16 kD, 14 and 23% for the rapid and slow cooling, respectively). This result, somewhat unexpected, we attributed to the presence of dioxane in swollen microspheres which could affect the crystallization process. In fact, removing dioxane from swollen microspheres proceeds parallel to crystallization with different speed, depending on actual mobility of polymer chains, parameters of solubility, etc. Effect of these processes on the degree of crystallinity was difficult to predict theoretically and detailed explanation of these phenomena would be tedious and of limited importance.

Annealing of microspheres in suspension (above T_g of poly(L-lactide)) was considered to be another promising way to achieve control over the degree of crystallinity of the microspheres. Annealing was performed in the following way. The required amount of concentrated suspension of microspheres in heptane was injected into the hot heptane and kept for the required time. Eventually, particles were rapidly cooled down to −30°C (ca. 80°C below T_g of poly(L-lactide) by mixing this suspension with excess of cold heptane. It was assumed that the temperature inside the small particles (microspheres with diameters close to 3 μm) was changed with a rate close to the rate of mixing.

The plot in Fig. 1 illustrates the relation between diameters of microspheres (determined by scanning electron microscopy) and time of their annealing at 80°C for particles with initial diameter $D_n = 2.49$ μm, diameter polydispersity $D_w/D_n = 1.17$, molecular weight $M_n = 7600$, and molecular weight polydispersity $M_w/M_n = 1.06$. From this plot it is possible to conclude that annealing of microspheres performed in the way described above does not change particle diameters.

In few experiments microspheres transferred to dodecane (four times repeated procedure of sedimentation of microspheres under gravitational forces followed by replacing the suspending medium with fresh portions of dodecane) were heated at 175°C. At these conditions microspheres were converted into droplets of liquid poly(L-lactide). After 20 min of heating this suspension was cooled down in the same way as suspension of microspheres annealed at 80°C. It is worth noting that this procedure resulted in coalescence of some particles into aggregates composed of two or three particles, however, their fraction did not exceed 3%. Diameters of the non-coalesced microspheres were found to remain unchanged.

Influence of the time of annealing of microspheres at 80°C onto molecular weight of constituting poly(L-lactide) is illustrated in Fig. 2. It was found that the molecular weight of poly(L-lactides) from annealed microspheres determined by GPC with RI detector did not change. Detection with multiangle light scattering detector (MALS) indicated some small increase of M_n which did not exceed 16% and was considered to be within the experimental error for low molecular weight samples.

Molecular weight of another sample, determined before and after the melting of particles, differed less than 6%, i.e. the difference was in the range of an experimental error. Storage of amorphous microspheres in suspension at room temperature over 2 years also did not change their molecular weight. Hence we could conclude that the described

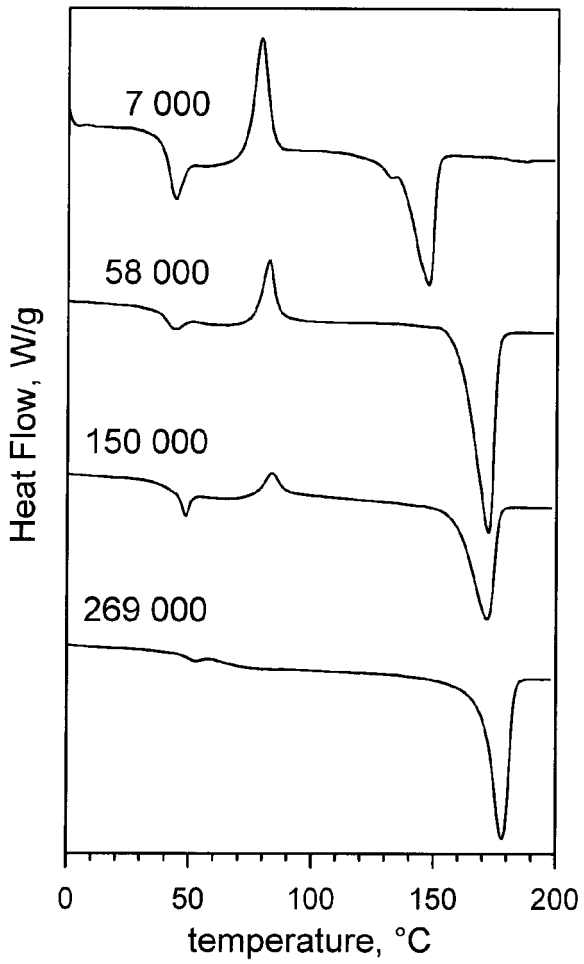

Fig. 3. DSC traces of native poly(L-lactide) microspheres composed of polymers with different molecular weight. Numbers indicate molecular weight of poly(L-lactide).

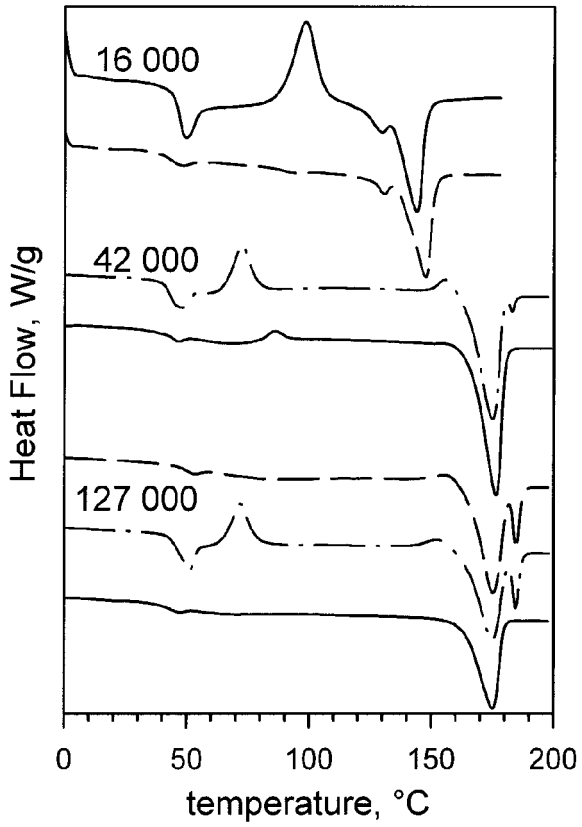

Fig. 4. Comparison of DSC traces of a native (solid line), melted (dashed–doted line), and annealed (dashed line) poly(L-lactide) microspheres. Numbers indicate molecular weight of poly(L-lactide).

procedures of annealing and/or melting of poly(L-lactide) microspheres do not change their diameters and molecular weight of the constituting polymers.

3.3. Crystallinity of the poly(L-lactide) microspheres

Crystallinity of the native and annealed poly(L-lactide) microspheres was determined by DSC and ^{13}C CP-MAS NMR methods. DSC curves of the native microspheres, obtained directly in polymerization (samples did contain traces of remaining monomer (<3%) and surfactant (<0.5%)) are shown in Fig. 3. These curves indicate the presence of a few characteristic transitions typical for semi-crystalline poly(L-lactide) samples (e.g. glass transition, cold crystallization, and melting) [16,21–29]. However, it is worth to note that in the earlier studies samples were not adequately characterized. Usually only one kind of averaged molecular weight was given (number, weight, or viscosity averaged) without information on polydispersity factor. In some instances the number average molecular weight and polydispersity factor were listed, however, calculated on the basis of polystyrene standards what was shown to be improper for poly(L-lactide) [30,31]. Majority of the investigated polymers had broad polydispersity. We present the data for poly(L-lactide)s that are much more uniform with respect to molecular weight, i.e. for polymers with relatively low molecular weight polydispersity factors ($M_w/M_n < 1.20$). Moreover, molecular weight was determined precisely using MALLS detector. On the other hand, our samples are composed of polymer dispersed on the micrometer scale. In these samples fraction of the boundary area (probably less ordered) is much higher than in the macroscopic ones.

Table 2
Dependence of the difference of enthalpy of melting and enthalpy of cold crystallization on molecular weight of poly(L-lactide) in microspheres

M_n (Da)	$\Delta H_m - \Delta H_c$ (J g^{-1})
7000	1.51
16,400	13.62
42,000	29.78
57,700	27.19
127,000	33.09
150,000	24.18
270,000	41.98

The following general relations were observed for

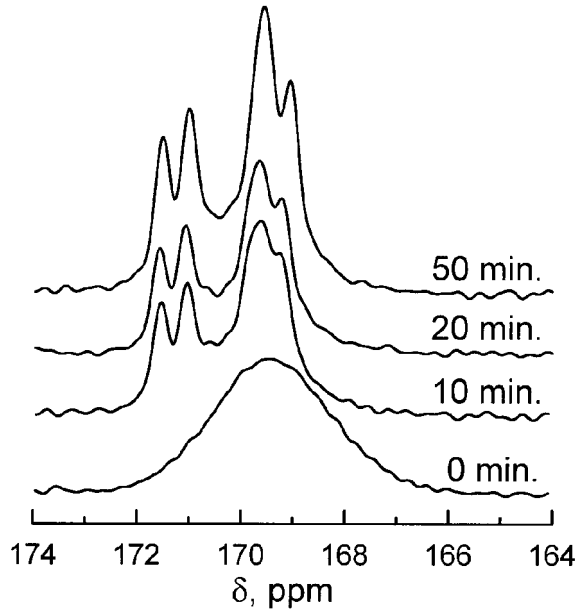

Fig. 5. Changes of signal of carbonyl carbon atom in ^{13}C CP-MAS NMR spectra of poly(L-lactide) microspheres annealed in suspension at 80°C.

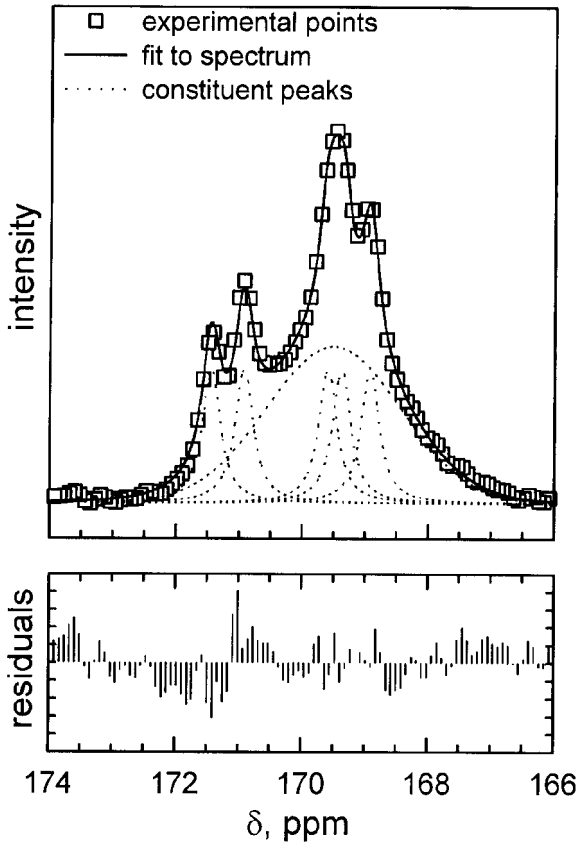

Fig. 6. Deconvolution of signal of carbonyl carbon atom into components due to amorphous and crystalline fractions.

thermal properties of nascent poly(L-lactide) microspheres (see Fig. 3). The glass transition was more efficiently pronounced for samples with lower molecular weight. Moreover, we noticed that the difference between the enthalpy of melting (ΔH_m) and the enthalpy of cold crystallization (ΔH_c), which is proportional to the degree of crystallinity, depended on the molecular weight of poly(L-lactide) in microspheres. The data characterizing this effect is given in Table 2.

DSC traces for the annealed and/or melted in suspension and rapidly solidified poly(L-lactide) microspheres are shown in Fig. 4. It is worth noting that for melted and rapidly solidified samples with M_n equal to 42 000 and 127 000 the signal of cold crystallization was more intensive and that inflection due to glass transition was better pronounced than for the parent original samples. These observations indicate that melting of poly(L-lactide) microspheres in suspension, followed by rapid quenching, yields particles with high content of amorphous fraction. Cold crystallization of these samples is manifested in DSC traces by two signals (observed also by Cohn [24] and Migliaresi [26]): the first one sharp with a maximum at 70°C and a long tail on the right-hand side and the second one which partially overlaps with the melting signal (at 172°C). The appearance of the second signal of cold crystallization is accompanied by the formation of the signal of melting at temperature equal to 183°C, i.e. at the temperature higher than for the main signal of melting (at 172°C). The presence of these signals suggests that the cold crystallization at high temperature lead to the formation of crystals with different structures (characterized by higher melting temperature). Such two distinct crystalline structures of poly(L-lactide) melting at different temperatures have been observed by Hoogsteen et al. [29], who examined hot-drawn poly(L-lactide) with high molecular weights ($M_v \sim 900$ kDa) [29].

Degree of crystallinity (χ_c) of poly(L-lactide) in microspheres can be determined from DSC measurements according to the following equation:

$$\chi_c = \frac{\Delta H_m - \Delta H_c}{\Delta H_m^0} \quad (1)$$

in which $\Delta H_m - \Delta H_c$ is the difference between the enthalpy of cold crystallization and the enthalpy of melting and ΔH_m^0 denotes the enthalpy of melting of 100% crystalline reference sample.

Shapes of the DSC traces shown in Figs. 3 and 4 indicated that precise determination of ΔH_m and ΔH_c was difficult because the high temperature portion of the signal of cold crystallization and the signal of melting do overlap. Moreover, the literature data for ΔH_m^0 vary significantly (from 93.6 [32] to 142 J g^{-1} [28]). In effect, values of χ_c obtained from DSC studies were characterized by substantial scattering. Thus, we looked for another more reliable and convenient method for the estimation of the degree of crystallinity of poly(L-lactide) in annealed microspheres.

It is known that ^{13}C CP-MAS spectra of amorphous and crystalline poly(L-lactide) are different and the signals of carbonyl carbon nucleus could be used for determining the

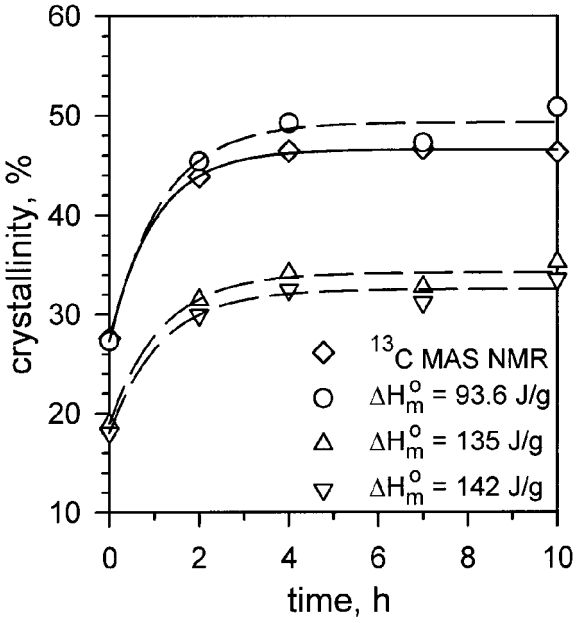

Fig. 7. Changes of the degree of crystallinity of poly(L-lactide) microspheres during annealing at 80°C.

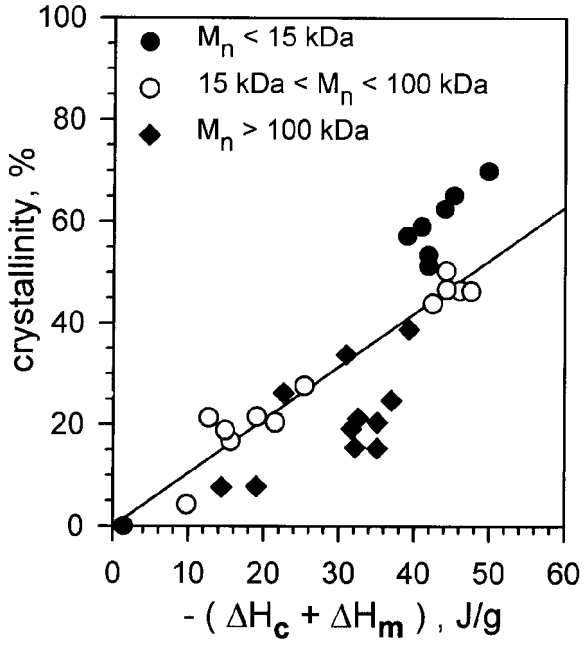

Fig. 8. Correlation of the degree of crystallinity determined by ^{13}C CP-MAS NMR with enthalpy of melting of crystalline fraction present in the native microspheres. Regression line calculated using samples with M_n in the range 15 kDa $< M_n <$ 100 kDa.

degree of crystallinity of poly(L-lactide) [23,33]. For amorphous polymers the signals are broad and structureless. For the crystalline ones the signals are much more narrow and with fine structure. However, until now the assignment of particular narrow signals to carbon atoms at various positions in crystal cells has not yet been made. The degree of crystallinity was evaluated by finding the contributions of spectra corresponding to the crystalline samples to the measured spectrum (best fit of a linear combination of spectra of amorphous and crystalline samples to the measured one).

Carbonyl signals in ^{13}C CP-MAS NMR spectra of poly(L-lactide) microspheres annealed in suspension, at 80°C, for various time periods are shown in Fig. 5. Five narrow lorenzian peaks, with the same amplitude and width, represented the crystalline fraction and the broad gaussian peak corresponded to the amorphous fraction. Signal shapes clearly indicated that samples annealed for the longer time periods were more crystalline. Fractions of amorphous and crystalline portions were determined by the integration of deconvoluted signals. Deconvolution into six signals was performed using the Marquardt–Levenberg algorithm. During fitting we did not impose any constrains on the chemical shifts and on the width of peaks. An example of fitting is shown in Fig. 6.

Comparison of the degree of crystallinity determined by DSC and by ^{13}C CP-MAS NMR methods for poly(L-lactide) microspheres annealed at 80°C for various time periods is shown in Fig. 7. Degree of crystallinity based on DSC measurements was calculated (according to Eq. (1)) using the experimentally determined values of ΔH_m and ΔH_c and values of ΔH_m^0 listed in the literature (93.6 [32], 135 [21], or 142 J g^{-1} [28]). The degree of crystallinity determined by ^{13}C CP-MAS NMR was calculated according to the description mentioned above. It was found that the best agreement between the values of crystallinity determined by ^{13}C CP-MAS NMR and by DSC was for ΔH_m^0 given by Fischer et al. ($\Delta H_m^0 = 93.6$ J g^{-1}) [32]. It is also worth noting that the values of χ_c determined by ^{13}C CP-MAS NMR are less scattered than those determined by the DSC suggesting higher reliability of the former method. According to Eq. (1) the difference $\Delta H_m - \Delta H_c$, being proportional to χ_c, is independent of ΔH_m^0, i.e. of the parameter values of which is reported in the literature vary significantly. Thus, in Fig. 8, we plotted χ_c determined by ^{13}C CP-MAS NMR as a function of $\Delta H_m - \Delta H_c$ obtained from DSC measurements. In the case of microspheres containing poly(L-lactide) with molecular masses in the range $15\,000 < M_n < 100\,000$ this dependence was approximated with a straight line crossing the origin of coordinates. Reciprocal of the slope of this line equals ΔH_m^0. Using the least square method we found $\Delta H_m^0 = 95$ J g^{-1} which was very close to ΔH_m^0 determined by Fischer et al. [32]. In Fig. 8 we also plotted the data for microspheres with $M_n < 15\,000$ and $M_n > 100\,000$. Points for these microspheres are most often located above (for $M_n < 15\,000$) and below ($M_n > 100\,000$) the line for polymers with M_n in the range from 15 000 to 100 000, indicating that there is some dependence of ΔH_m^0 on the molecular weight. Apparently, crystals composed of shorter macromolecules of poly(L-lactide) are less perfect, possibly due to the presence of numerous chain ends.

Our work was directed towards finding a convenient method allowing to control the degree of crystallinity of microspheres without affecting their diameters. Dependencies of the degree of crystallinity of microspheres made of

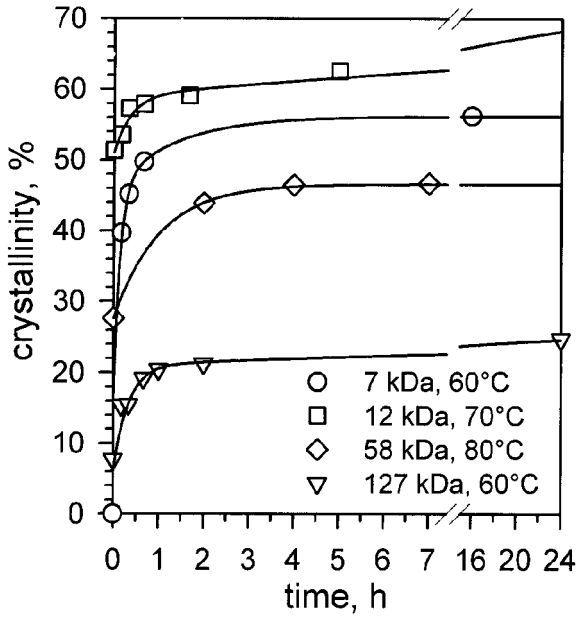

Fig. 9. Dependencies of the degree of crystallinity of poly(L-lactide) with different molecular weight on time of annealing.

poly(L-lactide) with various molecular masses on the time of annealing are shown in Fig. 9. It is worth to note that for microspheres composed of polymers with lower molecular masses, the degree of crystallinity can be controlled in the broader range. For example, for microspheres with $M_n = 7000$ the proper adjustment of time of annealing allowed to obtain particles with χ_c in the range from 0 to ca. 60%, whereas for microspheres made of polymer with $M_n = 127\,000$ values of χ_c could be regulated in the range from 0 to 21%.

While annealing of microspheres at 80°C allowed for increase of the degree of crystallinity of the parent particles, melting of highly crystalline microspheres in suspension, followed by rapid quenching at −78°C, yielded fully amorphous particles which could be further annealed to the required degree of crystallinity. Thus, by the appropriate thermal treatment it is possible to increase and decrease the degree of crystallinity of microspheres according to requirements.

4. Conclusions

Annealing and/or melting of poly(L-lactide) microspheres synthesized in ring-opening polymerization of L-lactide and suspended in hydrocarbons was found to be suitable for obtaining particles with controlled degree of crystallinity. The range in which the degree of crystallinity could be controlled was found to depend on the molecular weight of poly(L-lactide). For microspheres with $M_n = 7000$ the degree of crystallinity could be controlled in the range from 0 to 60%, whereas for microspheres with $M_n = 127\,000$ the degree of crystallinity could be varied only from 0 to 21%. Comparison of DSC and ^{13}C CP-MAS NMR methods used for the determination of the degree of crystallinity of poly(L-lactide) microspheres indicated that the latter one is more convenient and yields more reliable data.

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