

STUDY OF MORPHOLOGY AND MOLECULAR MOBILITY OF POLYHYDROXYBUTYRATE USING ^{13}C NMR SPECTROSCOPY

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1. Introduction

The most commonly used plastics are synthetic polymers derived from petrochemicals and so they tend to resist biodegradation. This leads to increasing pollution of environment with synthetic plastics waste what cannot be solved by traditional methods like solving, recycling or combustion. The way how to restrict further increasing of pollution is to use biodegradable plastics which are for a decade materials studied by many research groups.

The poly(3-hydroxybutyrate) (PHB) is the most studied biologically synthesised polymer which serves to prokaryotic and eukaryotic cells of microorganisms like energy storage [1]. It is synthesized by many bacteria [2, 3] and it is completely biodegradable into water and carbon dioxide [4, 5], biocompatible [6] and renewable, what predestines it for plenty applications for example drug delivering [7], biomedical materials [8], food packing and biodegradable plastics [9].

PHB is a linear optically pure polyester having the lamellar morphology with polymer chains perpendicular to the axis *c* and the amorphous phase formed between them [10]. The glass transition was found to be at 2 – 5 °C depending on crystal form, phase purity and polydispersity [11].

Common use of PHB is restricted by its high fragility with the 3 - 5 % total tensile elongation at break, poor thermal stability above its melting point and the strong effect of aging. The last mentioned phenomenon causes considerable changes of physical properties leading to increase of brittleness during the storage of material.

The simplest way of modification of this material is the thermal treatment below the melting temperature. The imperfect crystals with various temperature of melting (80 – 180 °C) are formed during uncontrolled crystallization of PHB leading to wider conformation distribution.

2. Experimental

For this research two samples were used. The first one was the virgin powdered PHB sample - PHB - and the second one was annealed in furnace for 48 hours at 80 °C - PHB(a).

The high-resolution solid-state NMR spectra were obtained on Varian 400 MHz NMR spectrometer (Palo Alto, CA, USA). The sample was put into ZrO_2 rotors with the diameter of 4 mm, centred inside by Teflon spacers and spun to frequency of 10 kHz at magic angle (MAS). The samples were measured at ambient temperature; the temperature inside the rotor during NMR measurement was 31°C [12].

The high-resolution single pulse ^{13}C MAS NMR spectra were measured at the resonance frequency of 100 MHz, free induction decay (FID) was recorded using the SPINAL pulse high-power proton decoupling of 90 kHz [13], the acquisition time and spectral width were set to 20 ms and 50 kHz, respectively. Each FID was an average of 1400

– 1500 scans to obtain well-resolved spectra with the recycle delay between two measurements 120 s and the final spectrum was obtained by Fourier transformation. The chemical shift was referenced to tetramethylsilane using adamantane as an external standard.

The spin-lattice relaxation times $T_1(^{13}\text{C})$ were obtained using ^1H - ^{13}C cross-polarization (CP) MAS NMR technique which was described in detail by D. A. Torchia [14]. All measurements were carried out at MAS rate of 10 kHz, with the time of the ^1H and ^{13}C $\pi/2$ pulses 2.8 and 4.8 μs , respectively. The CP Hartmann-Hahn condition was fulfilled with the radio frequency field strength of 60 kHz. The measurements were carried out at contact time of 2 ms, acquisition time of 20 ms and the delay time of 6 s, a pulse of high-power proton decoupling field of 90 kHz was applied during the data acquisition. Totally 256 scans were averaged to minimize the signal-noise ratio to measure the $T_1(^{13}\text{C})$ array spectra.

3. Results

The NMR spectra for both studied samples obtained using the single pulse ^{13}C MAS NMR at ambient temperature display four lines at 170.0, 68.5, 41.3 and 21.4 ppm which can be assigned to PHB carbons nuclei in four different groups - carbonyl (CO), methine (CH), methylene (CH_2) and methyl (CH_3) respectively (Figure 1). The ratios of areas beneath the particular lines differ from the expected 1:1:1:1 and they are 0.66:0.81:0.62:1.00 and 0.69:0.83:0.54:1.00 for virgin and annealed PHB, respectively.

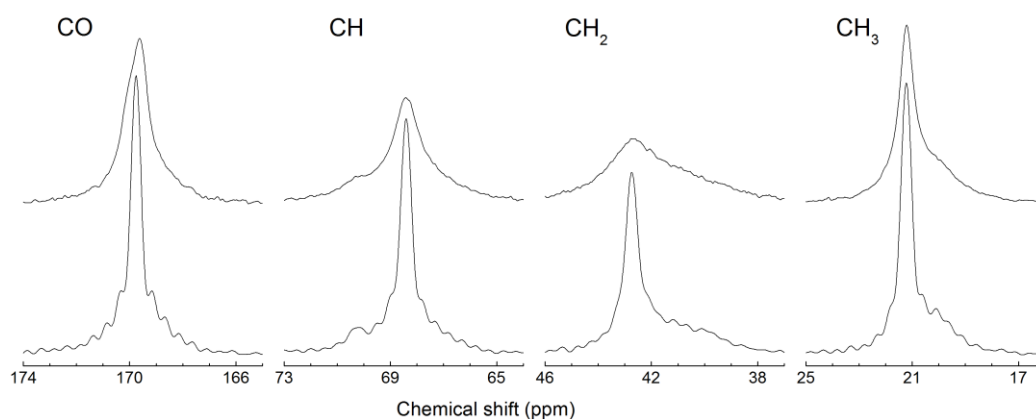


Fig. 1: Resonances observed in the single pulse ^{13}C MAS NMR spectra of virgin (bottom) and annealed PHB (top).

This difference indicates that the recycle delay of 120 s was long enough for methyl group but not for the other groups. For this reason only the signal of CH_3 carbon which is completely relaxed at the end of recycle delay group will be analysed. The asymmetrical shape of each NMR line is caused by the presence of two components– crystalline (narrow Lorentzian line) and amorphous (wide Gaussian line).

The methyl resonance was deconvoluted using MestReNova software with two lines assigned to carbons in crystalline and amorphous phase (Tab. 1). The crystallinity w of the samples was calculated as the ratio of the line intensity assigned to crystalline phase to the total area beneath methyl resonance. Annealing the PHB sample for two days at 80 °C led to the decrease in crystallinity from 75 % to 63 % which is caused by structural relaxation during the process of annealing, which could induce melting of larger imperfect crystals mentioned in the introduction. The increase of the widths of resonance lines for crystalline and amorphous component was also observed. This is connected with wider conformation distribution in annealed sample in comparison with the virgin PHB.

Tab. 1: *The weight fractions (w) and widths of peaks (Δf) resulting from deconvolution of the line related to the CH_3 groups.*

| | w_{cr} [%] | Δf_{cr} [Hz] | w_{am} [%] | Δf_{am} [Hz] |
|--------|------------------------|--------------------------------|------------------------|--------------------------------|
| PHB | 75 | 46 | 25 | 154 |
| PHB(a) | 63 | 67 | 37 | 209 |

The measurements of $T_1(^{13}\text{C})$ relaxation times were carried out changing the recovery time from 0.01 to 300 ms and detecting spectra. Each spectrum of the measured array was deconvoluted using Lorentzian and Gaussian lines assigned to crystalline and amorphous regions in the samples (Fig. 2 (a)). The positions of both lines were determined from the measured single pulse ^{13}C MAS NMR spectra. The decay of intensities as a function of increasing recovery time was fitted by mono-exponential function particularly for crystalline and amorphous component (Fig. 2(b)) for methyl group. The obtained long and short relaxation times from fitting procedure are in the Table 2.

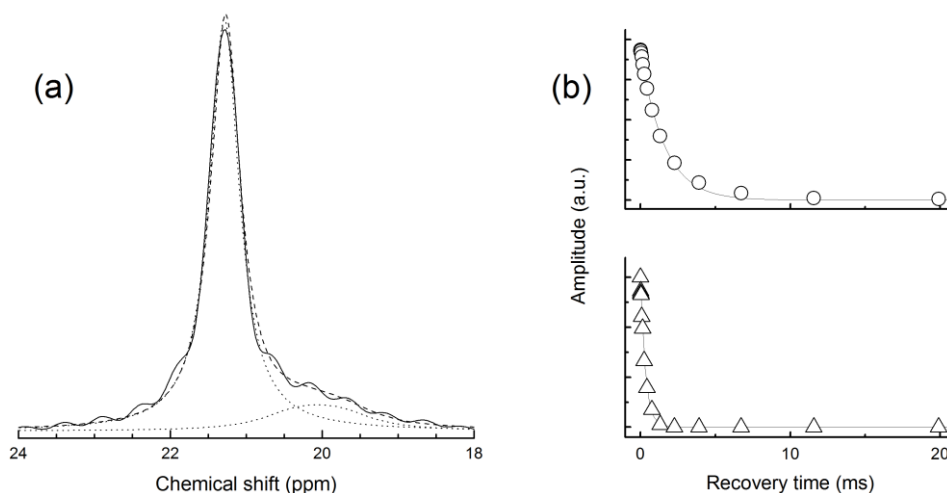


Fig. 2: *The analysis of $T_1(^{13}\text{C})$ relaxation times measurements. (a) Deconvolution of the spectrum obtained at 0.01 ms into two components. (b) The decrease in area related to the CH_3 carbon resonance versus the recovery time (symbols), exponential functions fitted to the data obtained for the crystalline (top) and amorphous (bottom) phase of virgin PHB (lines).*

The relaxation time depends not only on the strength of the magnetic interactions between ^{13}C and ^1H nuclei, but also on fluctuations in magnetic fields produced by the molecular motion of protons. Because of that, the relaxation time of CH_3 group is very short and its relaxation is also facilitated by the fact, that this group is a side group and has greater mobility. Significantly different relaxation times T_1 and T_s indicate the different mobility in crystalline and amorphous regions. Both values of relaxation time increase after annealing, which can result from the decrease in free volume in amorphous phase due to relaxation processes and increased space restrictions in crystalline phase.

Tab. 2: *The $T_l(^{13}\text{C})$ and $T_s(^{13}\text{C})$ spin-lattice relaxation times related to the crystalline (long) and amorphous (short) regions of the virgin and annealed PHB.*

| | | |
|--------|-----------|------|
| PHB | T_l (s) | 1.64 |
| | T_s (s) | 0.44 |
| PHB(a) | T_l (s) | 1.80 |
| | T_s (s) | 0.80 |

4. Conclusion

The single pulse ^{13}C MAS NMR measurements were carried out at ambient temperature on the virgin and annealed PHB samples. The analysis of the carbon methyl resonance confirmed, that both PHB systems are semicrystalline polymers. Annealing of the virgin PHB sample at 80 °C for 48 hours resulted in the crystallinity decrease from 0.75 to 0.63. The increase of the widths of the lines associated with the crystalline and amorphous regions due to broadening of conformation distribution was also observed. The long and short $T_1(^{13}\text{C})$ spin-lattice relaxation times associated with the crystalline and amorphous domains, respectively, were estimated and it was found out that annealing of the virgin PHB resulted in their increase.

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